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College of Graduate Studies

Sudan University of Science and Technology
College of Veterinary Medicine
Preventive Medicine and Public Health

Prevalence of Antibiotic Residues in Milk of Dairy Cattle in Khartoum state

انتشار متبقيات المضادات الحيويه في البان الأبقار بولاية الخرطوم

A Thesis Submitted to the College of Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Preventive Veterinary Medicine (MPVM)

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قال تعالى : (والذين جاهدوا فينا لنهدينهم سبلنا وان الله لمع المحسنين). العنكبوت (69).

Dedication

I dedicate this research first of all to the God who guide me to the success way in all my life

And my dear parents who light my life and support me all and the time , may god bless him

To my lovely brothers , sisters and their children

To all help , support and be beside me

With great love and thanks.

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مستخلص الدراسة

تم جمع عدد 236 عينات لبن عشوائيا من مزارع و مراكز التوزيع في ولايه الخرطوم خلال الفتره من اغسطس حتى سبتمبر 2015 م للتقصي عن مدى انتشار متبقيات المضادات الحيويه في البان الابقار .

تم استخدام اختبار Delvotest®SP كاختبار معياري للتقصي عن وجود المتبقيات الدوائيه.

تم الحصول على عدد 50 عينه ايجابيه بنسبه 21.18 % بالترتيب التالي 10 عينات ايجابيه بنسبه 20 % في محليه الخرطوم , 37 عينه ايجابيه بنسبه 74% في محليه بحري و 3 عينات ايجابيه بنسبه 6% في محليه امدرمان .

كان مجموع النسب المئويه الموجبه لعينات الحليب الفرديه 32.6 % بينما 15.6 % و 12.5 % من مراكز التوزيع وحافظات الحليب , بالترتيب .

كان اعلى متوسط لعينات الحليب الفرديه في محليه بحري بمتوسط 0.63 وانحراف معياري 0.48 يليها محليه ام درمان بمتوسط 0.14 وانحراف معياري 0.36 ثم محليه الخرطوم بمتوسط 0.05 وانحراف معياري 0.22 مع وجود فرق معنوي عالي (p-value < 0.000). يليها حافظات الحليب وكانت محليه الخرطوم الاعلى بمتوسط 0.21 وانحراف معياري 0.41 يليها محليه بحري بمتوسط 0.07 وانحراف معياري 0.27 ثم محليه ام درمان بمتوسط 0.04 وانحراف معياري 0.21 وكانت ال (p-value) < 0.140. وفي مراكز التوزيع كانت محليه الخرطوم الاعلى بمتوسط 0.40 وانحراف معياري 0.50 بينما كانت محليتي بحري و ام درمان المتوسط (0.00) والانحراف المعياري (0.00) على التوالي , مع وجود فرق معنوي عالي (p-value 0.000).

استخدم الاستبيان لجمع المعلومات عن استخدام الادويه والالمام والمعرفه بفتره سحب الدواء وتبين ان الاوكسي تتراسايكلين 20 % هو اكثر الادويه شيوعا في الاستخدام بضروره او بدون ضروره للعلاج به يليه البنسلين و التايلوسين مع عدم الالمام والدرايه بفترات السحب للمضادات الحيويه المستخدمه .

Abstract

A total of 236 milk samples were collected randomly from farms and sales points of Khartoum state during the period from August to September 2015 to investigate the prevalence of the antibiotic residues in dairy milk in Khartoum state.

Delvotest ®SP-ampule kits was used as standard test to investigate the presence of the drug residues.

The total positive result was found 50 samples at percentage of 21.18 % , as follows 10 samples (20 %) in Khartoum state , 37 samples (74%) in Bahry state and 3 samples only (6%) in Omdurman state.

Total of the positive percentage results from individual milk samples was 32.6 % while 15.6 % and 12.5 % from sales points and bulk milk sample ; respectively.

The high mean was found in the individual milk samples in (Bahry Mean : 0.63 ± STD: 0.48), (Omdurman Mean : 0.14 ± STD:0.36) and (khartoum Mean : 0.05 ± STD: 0.22) with high significant difference (p-value <0.000) . Then, in the bulk milk samples khartoum was the higest mean (khartoum Mean : 0.21 ± STD: 0.41) then (Bahry Mean : 0.07 ± STD: 0.27)and (Omdurman Mean : 0.04 ± STD:0.21) with (p-value< 0.140). Sales Points was higher in (khartoum Mean : 0.40 ± STD: 0.50) while Bahry and Omdurman was (Bahry Mean : (0.00) ± STD: (0.00), (Omdurman Mean : (0.00) ± STD: (0.00) respectively with high significant difference (p-value 0.000).

A questionnaire was used to collect data regarding to using drugs and acknowledge about withdrawal period reveled that oxytetracycline 20% is the most popular drug with or without any necessary to therapeutics then penicillin and tylosin and there is lack of knowledge to the withdrawal time of used antibiotics.

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Introduction

Consumption of milk is necessary to our life that's because it's primary source of nutrition for young mammals before they are able to digest other types of food. It contains many of nutrient's (calcium , magnesium , phosphorus , proteins , vitamins (A –C –B12- riboflavin) , iodine , zinc … etc.(Collet and Harrison.,1963)**.**There are two types of milk consumption: natural source of nutrition for all infant mammals and a food product for human of all ages that's derived from other animals. So that healthy life comes from healthy animals in the farm (Allison, 1985). The present study is concerned about the milk as a public health view, therefore when a cattle in a farm take the medicine by the owner or a veterinarian with lack of information about the dosage or withdrawal period time and the extensive use of the same drug, all these reasons lead to the chemical residues in the tissue, urine, fat and milk. It is known that the excretion of the drug is done by these routes but there are left over or trace amount of drugs called residues (Paige.*et .al*., (1997)), (Riviere and sundlof,(2009)). These residues cause many risks in the public health in human and the animal lives due to extensive use such as: resistance of the microbe against drugs, hypersensitivity, toxicity and cancers…etc. Otherwise these small amounts can remain in animal products and find their way into the food chain (Riviere, J. E., 1991) (Sundlof, S. F., 1994). The control of these residues can be calculated as maximum residue limits (MRLs) and acceptable daily intake (ADI) of the drug according to FDA, FAO, and Codex Almantaries (Fitzpatrick *et .al.,* 1995).Veterinary drugs are used in livestock to treat diseases, maintain herd and flock health, promote growth, improve meat and milk quality and increasing yield, otherwise reducing production costs (Richard, H. and Gustafson ,1990)**.** Some examples of the most used drugs in the field benzylepencillines, tetracyclines, ivermectines, sulphonamides, tylosin, gentamycine and phenylbutasone , which given in different formation by many routes such as I/V, I/M, I/Mm, S/C, Intravaginal/ intrauterine , orally...etc to treat several diseases like mastitis, pneumonia, parasitic infection and diarrhea. (Riviere, J. E., 1991).

Justification

In order to insure the uses of antibiotics is under the suitable and acceptable limits according to FAO\FDA.

Overall Objective

To determine the prevalence of antibiotics residues in dairy milk in Khartoum State.

Specific Objectives

1. To determine the antibiotics residues in diary milk in Khartoum state according to Delvotest®Sp-NT assay as screening test.
2. To identify the different types of antibiotics used for the treatment of bovine clinical conditions using structured questionnaire.

CHAPTER ONE

Literature review

"Veterinary drugs are any substance applied or administered to any food producing animal, such as cattle , poultry , fish or bees, whether used for theraputic, prophylactic or diagnostic purpose or for modification of physiological function or behavior " (IDF,1997).

1.1 Antibiotics and Antibiotics residues in milk

1.1.1:Definitions of Antibiotics:

Ammar,(2006) defined the Antibiotics as " Antibiotics are natural compounds produced mainly by microorganisms or are compounds obtained by chemical or microbiological modification of natural compounds and used to kill bacteria ."

Nawaz *et .al,* .(2001),defined Antibiotics as miracle drugs that are extensively used for the treatment and prevention of infectious disease in animals and humans. They concluded that antibiotics have greatly enhanced humans and animal's life , reduced mortality and improved quality of production.

"antibiotics are characterized by their powerful and specific action in the body against one or more types of bacteria ,low toxicity of tissues, stability and activity in the presence of body fluids with no destruction by certain tissue enzyme , too rapid excretion or development of resistant strains of microorganisms". (Singleton,1995).

"Antibiotics are low to medium molecular weight comounds exhibiting a variety of chemical and biological properties" (Chand Ram,2001).

1.1.2: Types of Antibiotics :

Antibiotics can be divided on the basis of their modes of action into four groups according to their mode of action ;

Those acting on the bacterial cell wall formation such as penicillins and bacitracin,those affecting the permeability of the cell membrane and leaking of cell contents including

polymixin and novobiocin; inhibing of protein synthesis by action on bacterial ribosome ; binding with the two nucleus protein 60S and 40S ribosomal units causing protein synthesis and misreading of the messenger RNA (m RNA) code resulting in production of abnormal polypeptides such as tetracyclines , aminoglycosides and erythromycin . Other act by inhibition of DNA eg. dependent RNA polymerase and blocking specific essential metabolic steps by antimetabolites such as rifamycin and enrofloxacin. (Alexander 1985 and Brander et al., 1991).

Table 1.1 : Shows the classification of antibiotics acording to the chemical structure (Heeschen,1991).

Table 1.1 : Antibiotics classification according to chemical structure :

Group	Internal group	Representative with importance
Carbohydrate antibiotics	1.Aminoglycoside antibiotics 2.Other (N- and C-) glycosides	Streptomycin, Neomycin
Macro cyclic lactone (lactam) antibiotics	1.Macrolide antibiotics 2.Polyene antibiotics ,3.Macrolactam antibiotics	Erythromycin Amphotericin
Quinone and similar antibiotics	_	Oligomycin
Amino acid Peptide antibiotics	_	Tetracyclines Penicillins, Cephalosporins, Bacitracin, Polymyxins
Nitrogen-containing Heterocyclic antibiotics	1.Non-condensed(single) heterocycles 2.Condensed (fused) heterocycles	No practical importance
Oxygen-containing Heterocyclic antibiotics	1.Furan derivatives 2.Pyran derivatives	No practical imporance
Alicyclic antibiotics	1.Cycloalkane derivatives 2.Small terpenes 3.Oligoterpene antibiotics	Streptovitacins
Aromatic antibiotics	1.Benzene compounds	Chloramphenicol

	2.Condensedaromatic comp 3.Non-benzene aromatic comp.	. Grisefulvin Novobiocin
Aliphatic antibiotics	1.Alkane derivatives 2.Aliphatic carbocyclic acid derivatives	Varitin

1.1.2.1: The Beta-lactam antibiotics :

They were described by Alexander (1985) as molecules including four-membered nitrogene atoms in each molecule and they acted by disrupting the synthesis of the cell wall envelope in growing bacterial cells and to destroy Beta-lactamase which affect the Beta lactam ring . Include: penicillin , benzylpenicillin , phenethicillin , methicillin , cloxacillin , ampicillin , amoxycillin , naphacillin , flucloxacillin , carbenicillin and other derivatives.

The basic structure of penicillins was found to consist of thiazolidine ring connected to a beta-lactam ring which attached a side chain (FAO,1991).

The benzylpenicillin molecular formula was given as C16H8N2O2S and called sodium salt of carboxilic acid. The rate of absorption , clearance and elimination of benzylpenicillin was reported by FAO(1991) to be influenced by the age of the recipient , formulations , route of administration , derivatisations especially as procaine benzylpenicillin and disease status. Benzylpenicillin was observed to be rabidly cleared from the blood through the kidney and execreated into urine unchanged.

A survey (Booth and Harding ,1986) in the UK showed that of3484 violations of antibiotic residues in milk recorded in 1984-85 almost 80% were caused by intramammary antibiotics and about 5% by injections in sites other than the udder, most of the remaining failures were for unknown reasons.
The majority of the treatments contained a penicillin and more than half the violations occurred following intramammary treatment of lactating cows and about 27% failures following treatment of dry cows shortly before Calving.

The main reasons for the violations were, failure to observe the withholding period for the milk (16.5%), accidental transfer of the milk (16.7%), prolonged excretion of antibiotic (8.2%) and early calving (7.3%).
In many countries there are more than 50 separate preparations containing a penicillin for the treatment of bovine mastitis and each formulation produces a different residue pattern in milk.

Since all preparations cannot be covered some studies recently done in France are cited as illustrations of the residues in milk.
Benzylpenicillin is widely used as a theraputic drug in all food animal speacies. It's especially used in the treatment of bovine mastitis in the lactating cow. Thus the residues in milk are concern.
The persistence of residue in milk depends on the formulation and route of administration. (Moretain and Boisseau 1984,1989).

1.1.2.2: Aminoglycosides :

Aminoglycosides were classified as bactericidal antibiotics . Their group contained amikacin, gentamycin , kanamycin, neomycin and streptomycin. They were found active mainly against Gram negative bacteria . (Singelton,1995).

Streptomycin was obtained from *streptomyces griseus* and chemically it consisted of nitrrous , base of streptidin and streptobiosamine (pyatkin and knvoshein,1980). Streptomycin was a base and forms salts with acids. Its salts are readily soluble in water but insoluble in organic solvents.

According to Alexander , 1985 streptomycin inhibited protein synthesis and transalation of genetic code. As it was highly polar compound streptomycin required active process in crossing the cell membrane . This involved electron transportation , oxidative phosphorylation and respiratory quinones in the cell membrane . Streptomycin is excreated by glomerular filtration in the kidney and about one-third of the drug is bound to and not pass glomerulus. Neomycin which was obtained from *streptomyces frachiae* was found to be water soluble compound similar to streptomycin in chemical structure , antimicrobial activities and pharmacology. It was poorly absorbed (2-10 %) from the intestinal tract in man or animal and was collected in the kidney and to a less extends in the liver . It was metabolized by phosphorylation , a denylation and acetylation in the digestive tract .

Absorbed neomycin was reported to be excreted via kidneys through glomeruler filtration (FAO, 1995).

An aminoglycoside gentamycin was produced by fermentation of *Micromonospora purpurea* (WHO,1995 B). It was a mixture of basic , water soluble compounds containing the aminocyclitol 2-dexystreptamine and two amino sugars. There were also minor components which might be present in formulations as the sulphate salt and used for the treatment of different bacterial infections in poultary and cattle . For food producing animal's combination with benzyl-penicillin , ampicillin and cloxacillin were used. It was poorly absorbed from gastrointestinal tract. Parental doses were distributed into the extracellular fluid , into the cortex of the kidney and the inner ear. Gentamycin residues were approximately 400 times high's in kidney cortex than in skeletal muscle . When administered to dogs and calves residues were shown to persist in kidney after 100 days after treatment.

The aminoglycoside spectinomycin was reported to inhibit the biosynthesis of protein (Alexander, 1985). However it was a bacteriostatic rather than bactericidal in action . It was used for the treatment of Gram-ve bacteria and mycoplasma . Other aminoglycosides included tobramycin which was administered with penicillins to control pseudomonas infection , framycetin which was used for the treatment of intestinal infections in calves and poultry.

1.1.2.3: The Tetracycline:

It was reported by singelton (1995) that tetracyclines acted by binding to ribosomes and inhibited the binding of aminoacyl-transfer ribo-nucleic acid (tRNA) to specific sites of the dioxyribo-nucleic acid (DNA).

These were discovered as a result of a search for antibiotics active against a wide range of bacteria than penicillins (Alexander, 1985). They were obtained from different species of streptomyces for example chlortetracycline obtained from *streptomyces aureofaciens* , oxytetracycline from *streptomyces rimosus* . They might be semi-synthetic like tetracycline , dimethyl-chloretetracycline , methacycline , boxycycline and minocycline . They inhibited a wider range of gram +ve and gram –ve bacteria than penicillin or streptomycin .

According to FAO (1991) , oxytetracycline was described as highly active and broad spectrum antibiotic . It was presented as amphoteric base compound , hydrochloride salts or as quaternary ammonium salt complex . The solubility of the base salt varied widely with pH. At 23°c it had minimum solubility at pH 5 of 500 mg/ml. the solubility rose to 31.40 mg/ml at ph 1.2 and 28 mg/ml at ph 9 . Oxytetracycline crystals did not lose their potency on heating for four days at 100°c . The amphoteric base of tetracycline formed salts with acids and bases. The hydrochloride salt was the most common form of oxytetracycline in parental and water-soluble animal health product . In pure state hydrochloride oxytetracycline crystals showed less than 5 % inactivation after 4 month storage at 50°c . Oxytetracycline was reported to be used in four major dosage product for animal health (FAO,1991). They might be feed premixes, which contain 50% oxytetracycline and more than 15 % quarterly ammonium compound and the remaining percentage was ash . The other forms were soluble powder, injectable solutions and tablets used for control of bacterial infections.

Due to limited metabolism of oxytetracycline, the determination of the parent compound in edible tissues by microbiological methods provides sufficient residue information for a safety assessment.

With the exception of small residues in kidney tissues, all tissues were
cleared of detectable levels of oxytetracycline within 5 days following dosage.Injectable forms of oxytetracycline yielded higher residues of oxytetracycline which persisted longer than oral dosage forms.

Extended withdrawal times are frequently required for long acting formulations of oxytetracycline.An ADI of 0-3 pg/kg of body weight does not permit recommending MRLs for edible tissues, milk and eggs that can be monitored with currently available microbiological methods of analysis.It is recommended that MRLs be established in milk, muscle, fat and eggs at thedetection level of the microbiological method: 0.1,0.1,0.1 and 0.2 mg/kg respectively in all species.Also, MRLs are recomended in liver and kidney of 0.3 and 0.6 mg/kg ,respectively in all species. The latter two recommendations reflect the typical residue distribution of oxytetracycline in these tissues.

The detection limit for oxytetracycline was 0.05 ppm. Levels as low as 1 ppb in bovine milk and meat have been attained using a tandem mass spectrometric approach (Traidi et al., 1985).

1.1.2.4: The Chloramphenicol :

This was described by Alexander 1985 , as broad spectrum antibiotic . it was first isolated as crystaline compounds from *streptomyces venezulae* . it had a simple chemical structure when compared with most of the other antibiotics and easily synthesized. Its solubility in water was found poor but it was soluble in organic solvents . in the body it is inactivated by conjugation with glucuronic acid , about one- third of the drug administered to the body was bound to plasma protein .

Chloramphenicol is bacteriostatic agent acting on the 50 S ribosomal subunits causing the inhibitions of transpeptidation in protein synthesis . This means that it blocks the transfer of aminoacids from tRNA to polypeptide chain on the ribosome and interferes with messenger ribonuclear acid (mRNA). (singelton, 1995) .

Chloramphenicol was reported by FAO ,(1994) to be rapidly absorbed whether administered via oral or parental route . The drug reached the maximum concentration in blood in 1-5 hours. Due to its low molecular weight , which was described less than 500 daltons. The major route of excretion in cattle was via the kidneys into the urine. Experiments showed that in 96 hours after administration in cattle the concentration in urine was 55.5% of the administered dose.

Thiamphenicol was an analogue of Chloramphenicol . It was a broad-specrum antibiotic and used for the control of infections in man , poultry and young calves. It was classified as bacteriostatic antibiotic against gram + and gram-ve bacteria . it was rapidly absorbed to differ from Chloramphenicol in that it was not metabolized ready in cattle ,poultry,sheep or human. It was excreated unchanged in the urine . (WHO,1998 A).

1.1.2.5: The polypeptide antibiotics :

These were described as a family of antibiotics. They included polymexin , bacitracin and neomomycin . polymyxins were obtained from *Bacillus polymyxa* . There were several polymyxins which were basic polypeptides. They were in the form of salts with mineral acids. These salts were water soluble and stable. Polymyxin B was the only member of polymyxins suitable for clinical use as it was the least toxic polymyxin. (Alexander , 1985).

Polymixins (colistin) are narrow spectrum antibiotics with activity against Gram negative organisms (Brander *et al.,* 1991). Polymyxin was reported to act by damaging the outer membrane and by increasing the permeability of the cytoplasmic membrane.(Singelton , 1995)

Bacitracin was polypeptide antibiotic which obtained from *Bacillus Subtilis* . It was active against many gm+ve bacteria . Its action by blocking the incorporation of nucleotides into cell wall. (Alexander , 1985) .

1.1.2.6: Sulphonamide and Trimethoprim :

Sulphonamides were antibacterial derived from sulphanilamide. (Dixon *et.al.,* 1993). Singelton, (1995) informed that Sulphonamides were synthetic bacteriostatic compounds used for control of wide range of gm+ve and gm –ve bacteria. They interfered with the synthesis of folic acid and coenzyme essential in a number of important metabolic reactions in bacteria cell. A Sulphonamide molecule was observed to be similar in shape to normal components of folic acid such as p-aminobenzoic acid . During folic acid synthesis sulphonamide might be inccorporated instead of p-aminobenzoic acid resulting in the formation of inactive analogue of folic acid .

Sulphonamides were absorbed from the gastrointestinal tract. Once absorbed they were bound to protein mainly to albumin . About 60-90% of the bounded protein was distributed to all tissues . The metabolism of sulphonamides was shown via N-acetylation . The product of metabolism had no antimicrobial activity. (Burtis and Ashwood ,1991).

Trimethoprim (Bushby,1983) an antibiotic which was used to complete the effect of Sulphonamides . It was found to inhibit the reduction of dihydrofolic acid to tetrahydrofolic acid (Brooks *et.al.,* 1995). The synergy between the Sulphonamides and Trimethoprim was first reported when used in the treatment of chicks infected with *plasmodium gallinaceum* . Sulphadimidine and its synonymes sulphamidine and sulphamezatnine were used to treat variety of bacteria disease in humans and food producing animals and for promoting growth of livestock . (FAO , 1990and WHO, 1995 A).

1.1.2.7: The Quinolones :

They were reported by (Renolds , 1993) as a group of synthetic antibiotics. This group included nalidixic acid , a naphthyridiene derivatives , cinoxacin , acinnoline derivatives , pipemidic and piromidic acids and pyridopyrimidine derivatives .

Nalidixic acid an active antibiotic against Gram positive bacteria due to its supercoiling activities of the gyrase (Singelton ,1995). The enzyme gyrase was found to be involved in DNA replication . bactericidal concentrations were achieved in urine , thus used in humans for treating urinary tract infections (Renolds ,1993).

Flumequine active mainly against Gram negative organism with antibacterial activity higher than Naldixic acid and more suitable for the treatment of systemic infections . Quinolones also included ciprofloxacine , enroxacin , homefloxacin , horfloxacin , oflaxacin , levoflaxacin , nadifloxacin , rufloxacin , sparfloxacin and tosufloxacin. (WHO,1995A).

1.1.2.8: The macrolides antibiotics :

They were a large family of antibiotics and were called macrolide because of their chemical structure which contained a large lactone ring (Alexander ,1985).They were described to be active against many Gram positive organisms and were bactriostatic and might be bactericidal depending on the number of bacteria and the concentration of the antibiotic . Their actions were found to be by inhibiting protein synthesis and binding the ribosomal sub-unit and interference with the transfer of amino acids from tRNA . The macrolide group was reported to contain erythromycin , spiramycin , oleanolomycin and tylosin . erythromycin was obtained from *streptomyces erythreus* and found to act by interfering with tRNA movement . It was reported to absorbed , distributed and excreted in urine and bile.

Spiramycin a macrolide antibiotic was produced by certain strains of *streptomyces ambofaciens* (WHO,1998 A). It was described to have a wide antibacterial activity and could persist longer in the tissuse and at a higher concentration will excreted in bile and faeces (Alexander ,1985).

Tylosin was widely used macrolide antibiotic because it was not very toxic it could absorbed well in the urine and bile . It was described to be active against Gram positive bacteria and a few Gram negative bacteria.

Tilimicosin was another macrolide antibiotic developed for veterinary use (WHO,1998 A). It was prepared in mixtures of cis and trans isomers in specific concentrations and was absorbed from gastrointestinal tract . Its absorption was observed to be in cattle than calves. More than 75% of the dose was eliminated within 7 days after administration with lesser amount in urine.

1.2 Hazard and Risk associated with Antibiotics residues in milk :

There are two types of hazards relating to drug residues direct short term hazards and indirect long term hazards. (Seri,2013).

-DIRECT AND SHORT TERM HAZARDS :

Drugs used in food animals can affect the public health because of their secretion in edible animal tissues in trace amounts usually called residues. For example, oxytetracycline (Salehzadeh et al., 2006) and enrofloxacin residues (Salehzadeh et al., 2007) have been found above the maximum residual level in chicken tissues. Similarly, diclofenac residues were reported to be the cause of vulture population decline in Pakistan (Oaks et al., 2004). Some drugs have the potential to produce toxic reactions in consumers directly; for example, clenbutarol caused illness in 135 peoples as a result of eating contaminated beef in Spain in 1990. Other types of drugs are able to produce allergic or hypersensitivity reactions. For example, 2-β lactam antibiotics can cause cutaneous eruptions, dermatitis, gastro-intestinal symptoms and anaphylaxis at very low doses. Such drugs include the penicillin and cephalosporin groups of antibiotics (Paige et al., 1997).

-INDIRECT AND LONG TERM HAZARDS :

Indirect and long term hazards include microbiological effects, carcinogenicity, reproductive effects and teratogenicity. Microbiological effects are one of the major health hazards in human beings. Antibiotic residues consumed along with edible tissues like milk, meat and eggs can produce resistance in bacterial populations in the consumers. This is one of the major reasons of

therapeutic failures amongst such peoples. Certain drugs like 3-nitrofurans and nitroimidiazoles can cause cancer in human population. Similarly, some drugs can produce reproductive and teratogenic effects at very low doses consumed for a prolonged period of time. One such example is vaginal clear cell adenocarcinoma and benign structural abnormalities of uterus with diethylstilbesterol (Sundlof, 1994).

Posssible health risk associated with Antibiotics residues in milk :

The possible adverce effects of antibiotic residues were reported to cause allergenic symptoms , disorders of intestinal flora and resistance of bacteria to antibiotic administered (Deiatowr, 1983).

These hazards and health risk include:

1.2.1: Allergenic effects :

Antibiotic residues had the capability to bind directly or indirectly to the protein of the final antigen (Deiatowr, 1983). Penicillin was a well-known example , the antibiotic or its metabolite penicillenic acid were also do bind to the amino acid lysine. The penicilloyl-protein conjugates were allergens. Cross immune-reactions which were neumerous between penicillin and different degradation products were also used to produce identical conjugates.

Senitization and allergic reactions were characterized by skin rashes and other unpleasant symptoms might occur in people already sensitive to a specific antibiotic (davis , 1986).

1.2.2: Disorders of intestinal flora :

Antibiotics resiues could affect the human intestinal flora and disturb it (archimbault ,1983). One of these disturbance was that the human intestines bacteria become resistant to antibiotics through prolonged consumption of law doses of antibiotic (fox and cameron ,1985).

1.2.3: Toxicological effects :

Antibiotic residues did not cause acute toxic effects due to their low quantity (archimbault ,1983). However scientific studies on toxicological risks were done for each substance . The joint FAO/WHO expert committee on food additives studied the relation between choloramphenicol and a plastic anemia and suggested that no alteration in incidence of

plastic anemia would occur due to choloramphenicol residues in food but it required more studies in this subject (WHO, 1995 A).

Studies in experimental animals for streptomycin and dihydrostreptomycin (WHO, 1995 B) showed that they were responsible for accumulation in the perilymph of the inner ear , renal damage , atoxia , anemia and impairment of hepatic function.

Many antibiotics caused acute toxicity to the host when administered in high dosage (Burtis *et . al.,* 1991). For example aminoglycosides could cause acute tubular necrosis when given in a dose more than 35 microgram per milliliter. Sulphonamides could produce crystalline aggregates in kidneys , ureters and bladder when given in a dose more than 125 microgram per milliliter.

1.2.4: Occurance of antibiotics resistance of bacterial strains :

Since 1960s , public health officials and scientists world wide had tried to quantity the role of antibiotics used in animals in bacterial resistance to antibiotics used for therapeutic purposes to treat human diseases (bonner,1997).

The antibiotics were reported by (lewis,1995) not to cause technically the resistance but allowed it to happen by creating a situation where an already existing variant could flourish. The use of antibiotic in livestock might lead to resistance to antibiotics.

1.3 Screening methodes for antibiotic residues detection :

Antibiotic residues in food of animal origin particulary milk are major food safety concern and require oractical methods for detecting , quantifing and identifying residues that may be present at levels above established safe residual limit (WHO,1997).

The test for drug residues in milk is performed by several methods such as:

1.3.1: bacterial growth inhibition assays.

1.3.2: chemical methods .

1.3.3: chromatographic analysis .

1.3.4: radioimmunological and enzyme technique .

1.3.5: electrophoresis methods .

1.3.1: Bacterial growth inhibition assays :

Bacterial growth inhibition methods were widely used as screening methods for detection of antibiotic residues . They had the advantage of being relatively inexpencive , rapid and permitted a large number of samples to be analyzed (Dixon *et.al,.* 1993).

A number of microbiological tests for detecting antibiotic residues have been developed as in 1941 , the cylinder plate assay method was first described between 1944 and 1945 ; the filter paper disc method was introduced(Bishop *et.al.,* 1992). Moreover , they mentioned that since the 1950s, the *Bacillus subtilis* disc assay method and modifications have been used to detect residual antibiotics in milk, and during the 1970s , the disc assay and the tube assay methods that use the *Bacillus stearothermophilus* organism gained acceptance and broad usage.

Inhibition methods relied on the presence of inhibiting substances affecting the growth of bacterial culture on the agar plate (Patal *et.al.,* 1996).

The limit of inhibition methods determination was set below the maximum residue limit (MRL) or as low as possible where no MRL had been set. It was described that in these methods the incidence of false positive results should be low.

The four plates test (FPT) was also known as the Frontier Post Test , it was a typical bacterial inhibition test (Dixon et.al., 1993). In this method discs of tissue were placed on four agar plates inoculated with microorganisms . the plates were incubated under different condition to allow inhibition of growth by a variety of anti microbial drugs. A positive result was indicated by complete inhibition of growth on the surface of the medium in a zone not less than 2 mm wide around the tissue disc.Other bacterial inhibition methods include the one plate test (OPT) which depends upon inhibiting *Bacillus subtilis* in agar medium containing trimethoprim for better detection of sulfonamides (Dierick *et al.,* 1995).

Other on farm methods were Delvotest-p and p-enzyme test using *Bacillus stearothermophilus* subspp. Calidolactis as test organism (Seymour *et al.,* 1988).*Bacillus subtilis* BGA *, Micrococcus luteus , Bacillus cerius* and *Sarcina lutea* were used for antibiotic residues testing (FAO ,1990).

1.3.2: Chemical methods :

They include high performance liquid chromatography (HPLC) and physical methods like mass spectroscopy which could provide more sensitive accurate and precise tests . They could differentiate between different antibiotics within a class such as the sulphonamides (Patal and Bond, 1996). One of the important difficulties that face (HPLC) was the need to different techniques to deal with different antibiotic classes . for example if a positive result was obtained from a microbial inhibition test and confirmation was needed , HPLC classes suspected should be carried out.

1.3.3: Radioimmunological and enzyme techniques :

Bishop et al., (1992) reported that during the late 1970 and 1980s, the development of radioimmunological and enzymes techniques for determining the presence of antibiotic residues took place. They also mentioned that during the 1980s, monoclonal antibody-based test that use antibodies for specific residues were introduced . (ELISA) was highly specific and easy to perform , benefiting from simple extraction procedures and reaction time (Patal and Bond 1996), as the result from ELISA were available in period less than one hour and large number of samples could be tested for antibiotic residues. Although , as they stated that wide ranges of ELISA tests were required to test for all possible antibiotic residues than microbial inhibition tests**.**

ELISA has become a fundamental tool for drug discovery , animal studies and clinical trials in pharmaceutical industry because of liability to assess large quantities of samples . In the pharmaceutical industry over 100.000 compounds are routinely tested using several different ELISA assays in nearly , drug screens to identify promising compounds (Stewart *et.al.,* 2005). The methods must be capable of high throughout performance with runs quickly and reliably performed in high volume. Therefore , variability and bias effects affect the system at several different levels :

1. The raw optical density readings.
2. The estimation of model parameters.
3. The estimation of analyzed concentration.

1.3.4: Electrophoresis methods :

High voltage electrophoresis bioautography was used for identification of sulphamethazine and penicillin in meat , milk and animal feed. They extracted the

antibiotic and performed electrophoresis using agar medium seeded with microorganism. Electrophoresis bioautography was used for determination of antibiotic residues in the tissue of slaughtered animals (Hardicka,1991).
Penicillin,streptomycine,neomycin,erythromycin,tylosin and tetracycline are identified *Bacillus subtilis* BGA, *B.sterothermophilus*, were the bacteria used for seeding the agar medium.

1.3.5: Chromatographic methods:

New chromatographic method had been introduced for determination of chloramphenicol in meat (Keukens *et.al.,*1992) and for determination of novobiocin residues in milk , blood and tisssue (Moats *et.al.,*1985). To quantify and confirm sulphamethazine residues in muscle and liver, liquid chromatography and a combination of gas chromatography and mass spectroscopy were used (Carignan and Carrier,1991).

1.4 Scientific definitions related to antibiotics residues monitoring :

The scientific terms related to monitoring antibiotic residues according to FAO ,1996 were acceptable dialy intake (ADI) for veterinary drug , bound residue , total residue , maximum residue limit of veterinary drug (MRLVD), withdrawal time and veterinary drug. (FAO ,1996).

1.4.1: Acceptable Dialy Intake (ADI) for Veterinary :

The Joint Expert Committee on Food Additives (JECFA) estimated ADI of a veterinary drug expressed on a body weight basis as the amount could be ingested dialy over a life time without appreciable health risk for a standard man weighting 60 kg. (FAO,1996).

1.4.2: Bound Residue :

The covalent binding of the parent drug or a metabolite of the drug and a cellular biological soluble or insoluble macromolecule was described by FAO 1996 to be bound residue which was not extractable from the macromolecule by exhausative extraction , denaturation or incorporation of metabolized radiolabelled fragments of the drug into endogenous compound or into the same macromolecule by normal pathways.

1.4.3: Total Residue of a Drug in Animals :

This was derived from food and consisted of the parent drug together with all the metabolites and drug based product (FAO,1996). They remained in the food after administration animals . The amount of total residues was determined by using radiolabelled drugs and was expressed as the parent equivalent in mg/kg of th food.

1.4.4: Maximum Residue Limit of Veterinary Drug (MRLVD) :

The maximum concentration of residues resulting from the use of a veterinary drug expressed in mg/kg or microgram/kg on a fresh weight basis which were recommended by Codex Almentarius Commission (CAC) to be legally permitted or recognized as acceptable in or on a food (FAO,1996).

MRLVD was based on the type and amount of residue considered to be without any toxicological hazard for human health expressed by the acceptable dialy intake (ADI). Also on the basis of a temporary ADI the MRL , VD could be reduced to the consistent with good practices in the use of veterinary drug and to the limit that practical analytical methods were available. Riviere and sundlof, 2001 defined it as the concentration of drug which if taken indefinitely by human will produce no ill effect . Table (1.2).

Table 1.2 : MRL of some veterinary drugs * :

ANTIBIOTIC	MRL
Benzyl penicillin	4
Ampicillin	4
Amoxycillin	4
Oxacillin	30
Cloxacillin	30
Dicloxacillin	30
Tetracycline	100
Oxytetracycline	100
Chlortetracycline	100
Streptomycin	200

Dihydrostreptomycine	200
Gentamycine	200
Neomycin	100
Sulphonamides	100
Trimethoprime	50
Spiramycin	200
Tylosine	50
Erythromycine	40
Quinalones	75
Polymyxine	50
Ceftiofur	100
Cefquinome	20
Nitrofurans	0
Nitromidazoles	0
Other chemotherapeutics (Chloramphenicol, Novobiocin)	0

*Nisha,2008.

1.4.5: Withdrawal Time :

This was described by FAO, 1996 as the period of time between the last administration of a drug and the collection of edible tissue or product from a treated animal that ensured the contents of residues in food were complying with MRLVD. WHO,2000 defined as this is the time which passes between the last dose given to the animal and the time when the level of residues in tissue (muscle , liver , kidney, skin and fat) or products (milk, eggs, homey) is lower than or equal to the MRL . Unless the withdrawal period has elapsed, the animal and its products must not be used for human consumption**.**

1.4.6: Veterinary Drug :

Accoding to FAO,1996 Veterinary drug was defined as the substance applied or administered to any food-producing animal whether used for theraputic purposes or for the modification of physiological functions or behavior. Any antibiotic used in

food producing animal for any of the a bove mentioned purposes was also defined as Veterinary drug.

1.4.7: MRLVD as settled by JECFA :

The Joint Expert Committee on Food Additives (JECFA) evaluated veterinary drug residues for setting acceptable dialy intake (ADI) and MRLVD it reviewed and updated previous setting levels according to information availablability concerning the specific antibiotic**.**

1.5 Withdrawel period or withholding time for some antibiotics:

Use of animal medicines is strictly controlled by law and requires observance of the withdrawal period . Table (1.3).

Table 1.3 : withdrawal period for some antibiotic are commonly used in the field gather from different companies (Sudan Index, 2013) :

Brand Name	Generic Name	Withdrawel Time	Manufacture	Country of Origin	Local Agent
Oxtra inj 5%	Oxytetracycline	15 days	Fatro Pharmaceutical Veterinary Industry	Italy	Badr veterinary Co.Ltd
Alamycin 5% inj	Oxytetracycline	15 days	Norbrook Laboratories Ltd.	Ireland	Alpha Medical Agencies Co.
Limoxin 200 LA inj	Oxytetracycline	7 days	Interchimie werken de adellar	The Netherlands	Alpha Medical Agencies Co.
Macrolan 200 inj	Tylosin	3 days	Interchimie werken de adellar	The Netherlands	Alpha Medical Agencies Co.
Tylosin 20%	Tylosin	4 days	United	Jordan	Zasco

inj			Veterinary Drugs Industrail Co. Ltd (UVDCo)		International Co.Ltd
Tylovit 20% inj	Tylosin	3 days	Pharma Swede	Egypt	Sudanese Canadian Company
Biocillin-150 LA inj	Amoxycillin	3 days	Interchimie werken de adellar	The Netherlands	Alpha Medical Agencies Co.
Betamox inj	Amoxycillin	After 24 hours from last treatment	Norbrook Laboratories Ltd.	Ireland	Alpha Medical Agencies Co.
Injectal 33% inj	sulphadimidine	3 days	Pharma Swede	Egypt	Sudanese Canadian Company
Noredine 24 inj	Sulphadimidine + Trimethoprim	48 hours	Norbrook Laboratories Ltd.	Ireland	Alpha Medical Agencies Co.
Co-trimoxazol 24% inj	Trimethoprim + Sulphamethazine	2 days	Kela Laboratoria N.V	Belgium	Agrimatco Co. Ltd
Vety-Enrox 10% inj	Enrofloxacine	7 days	Leads pharma (PVT)	pakistan	M&S for Human & veterinary Services
Floxad inj 10%	Enrofloxacine	14 days	Pharma Swede	Egypt	Sudanese Canadian Company
Gentaprim inj 30mg + 40mg+200 mg	Gentamycine Sulphate + Trimethoprim + Sulphamethoxin	4 days	Industrial Veterinaria S.A.INVESA	Spain	Agrimatco Co. Ltd

	e				
Genta-100 inj	Gentamycine Sulphate	4 days	Interchimie werken de adellar	The Netherlands	Alpha Medical Agencies Co.
Gentamycine 20% inj	Gentamycine Sulphate	3 days	Univet . Ltd	Ireland	Alrashideen for Investment Co.Ltd
Multiject imm	Procaine Penicillin +Streptomycin Sulphate+Neomycin Sulphate	72 hours	Norbrook Laboratories Ltd.	Ireland	Alpha Medical Agencies Co.
Terrexine imm 200 mg + 100,000 IU.	Cephalexin + Kanamycine	4-5 days	Univet . Ltd	Ireland	Alrashideen for Investment Co.Ltd
Gentamast 10 % imm	Gentamycine Sulphate	48 hours	Bremer Pharma GMBH	Germany	Apico Co.Ltd
Avimast imm	Oxytetracycline + Neomycine	4 days	Arab Veterinary Industrial Co. Ltd (AVICO)	Jordan	Avico Drugs & Investment Co.Ltd

1.6 Effect of heating on antibitic residues in milk :

Hapke and Grahwit (1987) approved that, the concentration of drug in animal tissue is directly correlated to the absorbed dose. The route of drug administration intramuscular or subcutaneous injection causes high concentration and persistence of drug residue at the site of injection (Standers *et.al.,* 1988). Sumano *et.al.,* 1990 concluded that, the drug clearance in healthy and diseased animal are not same . In diseased animals , residue could persist two or three time longer than in healthy animals. The influence of the drug residue level in tissues based on difference in absorption and deposition processes that vary between different animal species (Baggot ,1992). The oily preparation of drugs are delayed in clearance after local intramuscular injection .

The absence of inhibition zone around the penicillin disc from milk after boiling for 15 min. Explained that the penicillin antibiotic is affected by heat and denatured , but if mixed with milk , it may conjugate with milk protein and will not be denatured upon heating. The overall results of all experiments indicated that heating or boiling of milk will not cause disappearance of antibiotic residues and hence the risk of antibiotic contaminated milk exist even after boiling (Abdulrahman,2001).

In addition , Abdulrahman,2001 found that the absence of any effect of heating on tylosin , and very slight influence on oxytetracycline and penicillin in milk . The study indicated that there was aprobability of antibiotic hazard from contaminated milk to humans even after boiling.

High levels of natural inhibitors are present in mastitis milk and in colostrums and they can cause false positive resullts in the microbial growth inhibition assays (Kang and Kondo ,2001). Moreover , they found that false positive result in the Delvotest assay correlated with an increase in lactoferrin and lysozyme concentrations.

The heat treatment is a fast , simple and inexpensive method to remove false positive results as it has no effect on positive samples containing drugs . They also added that heat treatment before screening tests is an effective way to reduse false positive results in the milk samples.

CHAPTER TWO

Material and method

2.1 Study Area

Khartoum State (capital of Sudan) lies between longitudes 31.5 to 34° E and latitudes 15 to 16° N. (M.A.R.F.P., 2014).

It's the geographically divided into three provinces (Khartoum, Khartoum North (Bahry) and Omdurman) which are subdivided into seven localities as follows:

Khartoum (Jabal awlya locality, Khartoum locality), Khartoum North (Bahry) (East Nile locality, Bahry locality) and Omdurman (Umbada, Omdurman and karari localities).(Adel, E. and Omer,K.1999)

2.2 Study Design

The study design was a cross sectional study to investigate the prevalence of antibiotic residues in milk of dairy cattle in Khartoum state. (Michael thrusfild , 2006).

2.3 Sampling Method

A multistage simple random sampling method was adopted for milk samples collection from the different localities of Khartoum state as mentioned above in study area. (Michael thrusfild , 2006).

2.4 Sample Size:

The sample size was determined by simple random sampling method use 95% confidence interval and calculated by using the formula given by Martin (Martin *et. al.,* 1988).

$$N= 4\ PQ/L^2$$

P= expected prevalence was taken from previous study done in Khartoum (Ammar Mohamed, 2006) =38.9%

Q= 1-P = 1-38.9=37.9

L= allowable error = $(0.05)^2$

N= 4*38.9*37.9*10,000/100*100*25

N= 235.8 ~ 236 milk sample

2.5 Source of milk samples

236 bovine milk samples were collected during the period from 23 August to 23 September 2015 . The samples were sellected randomly from different localities of khartom state using multi stage cross-sectional method , from some farms ,containers and sales points as follows :

1. 83 samples were collected from farms ,containers and sales points in Khartoum north (east nile, kuku , shambat , kadaru and al maona street) as follows :
   - 41 samples from cows
   - 26 samples from containers
   - 16 samples from sales points

   (table 2.1)

2. 95 samples were collected from farms ,containers and sales points in Khartoum (burri, soba al hella , al salama and jabal awlia) as follows :
   - 37 samples from cows
   - 33 samples from containers
   - 25 samples from sales points

   (table 2.1)

3. 58 samples were collected from farms ,containers and sales points in Omdurman (om bada, moweleh and al thawrat) as follows :
   - 14 samples from cows
   - 21 samples from containers
   - 23 samples from sales points

   (table 2.1)

Table 2.1 : Sampling maner :

locality	Districs	Area	Individuals milk	Bulk Milk	Sales points	Total
1- Khartoum Bahry	1.east nile	Kuku	24	6	10	40
	2.shambat	Sh.agriculture	17	8		25
	3.bahry	Al-maona street	_	_	6	6
	4.al-kadaru	alseleet	_	12	_	_
Total						83
2- Khartoum	1.Burri/ al manshia	_			10	10
	2. soba	Al hella	_	_	15	15
	3.saffola / al salama			30		30
	4.jabal awlyaa	Wad naeem/ shegelab	37	3		40
Total						95
3- Omdurman		Ombadda , al thawrat, al moweleh	14	21	23	58
Total			92	80	64	236

2.6 Collection of the samples

The samples were collected into clean sterile plain bottles and cooled immediately at approximately 4-5°c in an ice bag until examinations were carried out . The samples were

transported to the microbiology laboratory of the SUST college of veterinary medicine for analysis.

2.7 Detection of antibiotic residues in milk

Antibiotic residues were determined using Delvotest® SP-ampule Kit (202- Delvotest SP 100 , 3 test box , DSM Food specialties , the Netherlands).

2.7.1 Delvotest® SP-ampule :

Delvotest was the standard diffusion test for the detection of antibacterial substances in milk , in addition to detecting the antibiotics at the safe or tolerance levels (Bishop *et al.,* 1992).

The test is based on the rapid growth and acid production of *Bacillus stearothermophilus var . calidolactis* with the associated subsequent change in the bromocresol purple dye from purple to yellow (Bishop *et al.,* 1992).

2.7.1.1: principle of the test :

The principle for this test based on comparing clear zones on an agar plate medium to which bacterial spores have been seeded. Zones that belong to samples are compared with the zones of known amount of penicillin for quantitative determinations. Sensitivity and reproducibility of the method is affected by the depth of agar , where a thin layer is more sensitive than a thick layer. Acid production during growth of *Bacillus stearothermophilus var . calidolactis* is utilized to develop commercial Delvotest SP (DSM, Netherlands.) if inhibitors are absent , the bacteria grow and produce acid , a change is seen in the indicator . Test kits are available for individual as well as for multiple sample analysis.(Hui, Y.H., 1993a). (Table Appendix G).

2.7.1.2 : Equipment :

1- An ampule with *Bacillus stearothermophilus var . calidolactis* in solid agar medium with bromocresol purple (0.025 mg) and nutrient tablets composed of tryptone (0.5 mg), glucose (5 mg) and non fat dry milk (2 mg).

2- Disposable tips to transfer (0.1 ml) of milk sample to ampule.

3- Incubator set at 64°c (±2°c) for three hours.

2.7.1.3: Procdure :

The required number of ampoules were cut off and opened by punching a hole in the aluminum foil and mark it. One nutrient table was added to all ampules by tweezers. A fresh disposable pipette tip was placed onto the syringe and 0.1 ml milk sample was transfered into the ampoule. The ampoules were incubated in dry incubator at 64°c (± 2°c) for three hours.

2.7.1.4: Interpretation of the result :

Result were read out from the lower two thirds of the agar medium as follows :

- Yellow colour indicated absence of antibiotic residues .
- Purple colour indicated presence of antibiotic residues .
- A Yellow / Purple colour indicated the presence of antibiotic residues in concentrations near the detection limit (false positive). **(see AppendixE).**

2.8 Statistical Analysis

The analysis of results were carried out using Statistical Package for Social Science program (SPSS) version 16.0. Descriptive statistical analysis was displayed in frequency distribution and cross tabulation tables. Univariate analysis using chi-square for qualitative data describe the risk factors , number of tested and degree of freedoms. Chi – square at p-value ($p<0.25$) was considered as significant association and the risk factors was selected to enter the multivariate analysis. Multivariate logistic regression model was used to describe the risk factors , number positive, odds ratio , CI 95% and p-value at ($p<0.05$) indicated significant association between the result and different municipalities of Khartoum state . One WAY ANOVA was performed statistic significant was set at a p-value of (≤ 0.05) to determine the association between the different localities and other groups (individual, bulk and sales points milk samples) which of them highest or lower detected about antibiotic residues.

CHAPTER THREE

Result

A total of 236 milk samples were collected using structured questionaire (**Appendix F)** to gather infaormation from different dairy farms , bulk milks and sales points from three localities of Khartoum state (khartoum , Bahry and Omdurman). Table (3.1).

Table 3.1 : The result of the milk samples:

No. of sample	Type	source	result
1	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
2	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
3	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
4	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
5	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
6	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
7	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
8	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
9	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
10	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve

11	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
12	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
13	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
14	Individual milk sample	Mahlab kuku – bahry (farm 1)	+ ve
15	Bulk milk sample	Mahlab kuku – bahry (container 1)	+ ve
16	Bulk milk sample	Mahlab kuku – bahry (container 2)	+ ve
17	Bulk milk sample	Mahlab kuku – bahry (container 3)	+ ve
18	Bulk milk sample	Mahlab kuku – bahry (container 4)	+ ve
19	Bulk milk sample	Mahlab kuku – bahry (container 5)	+ ve
20	Bulk milk sample	Mahlab kuku – bahry (container 6)	+ ve
21	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
22	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
23	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
24	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
25	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
26	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
27	Individual milk sample	SUST farm - kuku –	+ ve

		bahry (farm 2)	
28	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
29	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
30	Individual milk sample	SUST farm - kuku – bahry (farm 2)	+ ve
31	Sales points milk sample	East Nile centers (center 1)	+ ve
32	Sales points milk sample	East Nile centers (center 2)	- ve
33	Sales points milk sample	East Nile centers (center 3)	+ ve
34	Sales points milk sample	East Nile centers (center 4)	+ ve
35	Sales points milk sample	East Nile centers (center 5)	+ ve
36	Sales points milk sample	East Nile centers (center 6)	+ ve
37	Sales points milk sample	East Nile centers (center 7)	+ ve
38	Sales points milk sample	East Nile centers (center 8)	+ ve
39	Sales points milk sample	East Nile centers (center 9)	+ ve
40	Sales points milk sample	East Nile centers (center 10)	+ ve
41	Individual milk sample	shambat agriculture farms – bahry (farm 3)	- ve
42	Individual milk sample	shambat agriculture farms – bahry (farm 3)	- ve

43	Individual milk sample	shambat agriculture farms – bahry (farm 3)	- ve
44	Individual milk sample	shambat agriculture farms – bahry (farm 3)	- ve
45	Individual milk sample	shambat agriculture farms – bahry (farm 3)	- ve
46	Individual milk sample	shambat agriculture farms – bahry (farm 4)	- ve
47	Individual milk sample	shambat agriculture farms – bahry (farm 4)	- ve
48	Individual milk sample	shambat agriculture farms – bahry (farm 4)	- ve
49	Individual milk sample	shambat agriculture farms – bahry (farm 4)	- ve
50	Individual milk sample	shambat agriculture farms – bahry (farm 5)	- ve
51	Individual milk sample	shambat agriculture farms – bahry (farm 5)	- ve
52	Individual milk sample	shambat agriculture farms – bahry (farm 5)	- ve
53	Individual milk sample	shambat agriculture farms – bahry (farm 5)	+ ve

54	Individual milk sample	shambat agriculture farms – bahry (farm 6)	- ve
55	Individual milk sample	shambat agriculture farms – bahry (farm 6)	- ve
56	Individual milk sample	shambat agriculture farms – bahry (farm 6)	+ve
57	Individual milk sample	shambat agriculture farms – bahry (farm 6)	- ve
58	Bulk milk sample	shambat agriculture – bahy (container 7)	-ve
59	Bulk milk sample	shambat agriculture – bahy (container 8)	-ve
60	Bulk milk sample	shambat agriculture – bahy (container 9)	-ve
61	Bulk milk sample	shambat agriculture – bahy (container 10)	-ve
62	Bulk milk sample	shambat agriculture – bahy (container 11)	+ve
63	Bulk milk sample	shambat agriculture – bahy (container 12)	-ve
64	Bulk milk sample	shambat agriculture – bahy (container 13)	-ve
65	Bulk milk sample	shambat agriculture – bahy (container 14)	-ve
66	Sales points milk sample	Al ma'ona street centers – bary (center 11)	- ve
67	Sales points milk sample	Al ma'ona street centers – bary	- ve

		(center 12)	
68	Sales points milk sample	Al ma'ona street centers – bary (center 13)	- ve
69	Sales points milk sample	Al ma'ona street centers – bary (center 14)	- ve
70	Sales points milk sample	Al ma'ona street centers – bary (center 15)	- ve
71	Sales points milk sample	Al ma'ona street centers – bary (center 16)	- ve
72	Bulk milk sample	Al Kadaru- al sellt – bahy (container 15)	-ve
73	Bulk milk sample	Al Kadaru- al sellt – bahy (container 16)	-ve
74	Bulk milk sample	Al Kadaru- al sellt – bahy (container 17)	-ve
75	Bulk milk sample	Al Kadaru- al sellt – bahy (container 18)	-ve
76	Bulk milk sample	Al Kadaru- al sellt – bahy (container 19)	-ve
77	Bulk milk sample	Al Kadaru- al sellt – bahy (container 20)	-ve
78	Bulk milk sample	Al Kadaru- al sellt – bahy (container 21)	-ve
79	Bulk milk sample	Al Kadaru- al sellt – bahy (container 22)	-ve
80	Bulk milk sample	Al Kadaru- al sellt – bahy (container 23)	-ve
81	Bulk milk sample	Al Kadaru- al sellt – bahy (container 24)	-ve

82	Bulk milk sample	Al Kadaru- al sellt – bahy (container 25)	-ve
83	Bulk milk sample	Al Kadaru- al sellt – bahy (container 26)	-ve
84	Sale points sample	Burri / al manshea centers – Khartoum (center 1)	-ve
85	Sale points sample	Burri / al manshea centers – Khartoum (center 2)	-ve
86	Sale points sample	Burri / al manshea centers – Khartoum (center 3)	+ve
87	Sale points sample	Burri / al manshea centers – Khartoum (center 4)	-ve
88	Sale points sample	Burri / al manshea centers – Khartoum (center 5)	-ve
89	Sale points sample	Burri / al manshea centers – Khartoum (center 6)	-ve
90	Sale points sample	Burri / al manshea centers – Khartoum (center 7)	-ve
91	Sale points sample	Burri / al manshea centers – Khartoum (center 8)	-ve
92	Sale points sample	Soba al hella centers – Khartoum (center 9)	-ve
93	Sale points sample	Soba al hella centers – Khartoum (center	-ve

		10)	
94	Sale points sample	Soba al hella centers – Khartoum (center11)	-ve
95	Sale points sample	Soba al hella centers – Khartoum (center12)	-ve
96	Sale points sample	Soba al hella centers – Khartoum (center 13)	-ve
97	Sale points sample	Soba al hella centers – Khartoum (center 14)	-ve
98	Sale points sample	Soba al hella centers – Khartoum (center 15)	-ve
99	Sale points sample	Soba al hella centers – Khartoum (center 16)	-ve
100	Sale points sample	Soba al hella centers – Khartoum (center 17)	-ve
101	Sale points sample	Soba al hella centers – Khartoum (center 18)	-ve
102	Sale points sample	Soba al hella centers – Khartoum (center 19)	-ve
103	Sale points sample	Soba al hella centers – Khartoum (center 20)	-ve
104	Sale points sample	Soba al hella centers – Khartoum (center	-ve

		21)	
105	Sale points sample	Soba al hella centers – Khartoum (center 22)	-ve
106	Sale points sample	Soba al hella centers – Khartoum (center 23)	-ve
107	Sale points sample	Soba al hella centers – Khartoum (center 24)	-ve
108	Sale points sample	Soba al hella centers – Khartoum (center 25)	-ve
109	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 1)	-ve
110	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 2)	-ve
111	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 3)	-ve
112	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 4)	-ve
113	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 5)	-ve

114	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 6)	-ve
115	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 7)	-ve
116	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 8)	-ve
117	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 9)	-ve
118	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 10)	-ve
119	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 11)	-ve
120	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 12)	-ve
121	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 13)	-ve
122	Bulk milk sample	Saffola/ al ssalama	+ve

		containers – Khartoum (container 14)	
123	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 15)	-ve
124	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 16)	-ve
125	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 17)	-ve
126	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 18)	-ve
127	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 19)	-ve
128	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 20)	-ve
129	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container21)	-ve
130	Bulk milk sample	Saffola/ al ssalama containers –	-ve

		Khartoum (container 22)	
131	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 23)	+ve
132	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 24)	-ve
133	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 25)	-ve
134	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 26)	-ve
135	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 27)	-ve
136	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 28)	-ve
137	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container 29)	-ve
138	Bulk milk sample	Saffola/ al ssalama containers – Khartoum (container	-ve

		30)	
139	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 1)	-ve
140	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 1)	-ve
141	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 1)	-ve
142	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 1)	-ve
143	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 1)	-ve
144	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 2)	-ve
145	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 2)	-ve
146	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 2)	-ve
147	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 3)	-ve
148	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 3)	+ve
149	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 3)	-ve
150	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 4)	-ve
151	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 4)	-ve
152	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 4)	-ve
153	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 4)	-ve
154	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 4)	-ve

155	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 4)	-ve
156	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 4)	-ve
157	Bulk milk sample	Jabal awlyaa containers – Khartoum (container 31)	-ve
158	Bulk milk sample	Jabal awlyaa containers – Khartoum (container 32)	-ve
159	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
160	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	+ve
161	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
162	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
163	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
164	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
165	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
166	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
167	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
168	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 5)	-ve
169	Individual milk sample	Jabal awlyaa farms –	-ve

		Khartoum (farm 6)	
170	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
171	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
172	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
173	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
174	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
175	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
176	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
177	Individual milk sample	Jabal awlyaa farms – Khartoum (farm 6)	-ve
178	Bulk milk sample	Jabal awlyaa containers – Khartoum (container 33)	-ve
179	Sales points sample	Omdurman centers (center 1)	-ve
180	Sales points sample	Omdurman centers (center 2)	-ve
181	Sales points sample	Omdurman centers (center 3)	-ve
182	Sales points sample	Omdurman centers (center 4)	-ve
183	Sales points sample	Omdurman centers (center 5)	-ve
184	Sales points sample	Omdurman centers (center 6)	-ve

185	Sales points sample	Omdurman centers (center 7)	-ve
186	Sales points sample	Omdurman centers (center 8)	-ve
187	Sales points sample	Omdurman centers (center 9)	-ve
188	Sales points sample	Omdurman centers (center 10)	-ve
189	Sales points sample	Omdurman centers (center 11)	-ve
190	Sales points sample	Omdurman centers (center 12)	-ve
191	Sales points sample	Omdurman centers (center 13)	-ve
192	Sales points sample	Omdurman centers (center 14)	-ve
193	Sales points sample	Omdurman centers (center 15)	-ve
194	Sales points sample	Omdurman centers (center 16)	-ve
195	Sales points sample	Omdurman centers (center 17)	-ve
196	Sales points sample	Omdurman centers (center 18)	-ve
197	Sales points sample	Omdurman centers (center 19)	-ve
198	Sales points sample	Omdurman centers (center 20)	-ve
199	Sales points sample	Omdurman centers (center 21)	-ve
200	Sales points sample	Omdurman centers (center 22)	-ve
201	Sales points sample	Omdurman centers	-ve

		(center 23)	
202	Individual milk sample	Omdurman farms (farm 1)	-ve
203	Individual milk sample	Omdurman farms (farm 1)	-ve
204	Individual milk sample	Omdurman farms (farm 1)	-ve
205	Individual milk sample	Omdurman farms (farm 1)	-ve
206	Individual milk sample	Omdurman farms (farm 1)	-ve
207	Individual milk sample	Omdurman farms (farm 1)	-ve
208	Individual milk sample	Omdurman farms (farm 1)	+ve
209	Individual milk sample	Omdurman farms (farm 2)	-ve
210	Individual milk sample	Omdurman farms (farm 2)	-ve
211	Individual milk sample	Omdurman farms (farm 2)	-ve
212	Individual milk sample	Omdurman farms (farm 2)	-ve
213	Individual milk sample	Omdurman farms (farm 2)	-ve
214	Individual milk sample	Omdurman farms (farm 2)	+ve
215	Individual milk sample	Omdurman farms (farm 3)	-ve
216	Bulk milk sample	Omdurman containers (container 1)	-ve
217	Bulk milk sample	Omdurman	-ve

		containers (container 2)	
218	Bulk milk sample	Omdurman containers (container 3)	-ve
219	Bulk milk sample	Omdurman containers (container 4)	-ve
220	Bulk milk sample	Omdurman containers (container 5)	-ve
221	Bulk milk sample	Omdurman containers (container 6)	-ve
222	Bulk milk sample	Omdurman containers (container 7)	-ve
223	Bulk milk sample	Omdurman containers (container 8)	-ve
224	Bulk milk sample	Omdurman containers (container 9)	-ve
225	Bulk milk sample	Omdurman containers (container 10)	-ve
226	Bulk milk sample	Omdurman containers (container 11)	-ve
227	Bulk milk sample	Omdurman containers (container 12)	-ve
228	Bulk milk sample	Omdurman	-ve

		containers (container 13)	
229	Bulk milk sample	Omdurman containers (container 14)	-ve
230	Bulk milk sample	Omdurman containers (container 15)	-ve
231	Bulk milk sample	Omdurman containers (container 16)	-ve
232	Bulk milk sample	Omdurman containers (container 17)	-ve
233	Bulk milk sample	Omdurman containers (container 18)	-ve
234	Bulk milk sample	Omdurman containers (container 19)	+ve
235	Bulk milk sample	Omdurman containers (container 20)	-ve
236	Bulk milk sample	Omdurman containers (container 21)	-ve

Frequency tables shows the informations from 15 farms selected randomely from different localities in Khartoum State that mentioned above.

Theileriosis 4(26.7%) is the most popular disease in the farms then pneumonia, mastitis and meningeoencephalitis (3(20%) , 3(20%) and 1(6.7%)) respictively. And Oxytetracyclines 20% 5(33.3%) is the most popular drugs then penicillin , tylosin with sulpha, oxy with other drugs,

tylosin , tylosin with penicillin and puparvacone with other drugs like phenylbutazone and vitamines (2(13.3%) , 2(13.3%) , 2(13.3%) , 1(6.7%) , 1(6.7%) and 1(6.7%) respictively. 11(73.3%) doesn't milking during theraputics while 4(26.7%) do that.

From 13 (86.7%) know about withdrawal period , 13 (86.7%) they discard the milk while 2 (13.3%) selling for consumption and 2 (13.3%) they unkown about the withdrawal period and don't care.

(Tables 3.2 , 3.3 , 3.4 & 3.5).

Table 3.2 : frequency table of farms in Khartoum State :

Risk factors	Frequency	percent	Cumulative percent
Herd size:			
-small	5	33.3	33.3
-medium	8	53.3	86.7
-large	2	13.3	100
Total	15	100	
Health status :			
-poor	1	6.7	6.7
-good	14	93.3	100
Total	15	100	
Most popular disease :			
-meningeoencephalitis			
-pneumonia			
-mastitis	1	6.7	6.7
-theileriosis	3	20	26.7
-pneumonia & mastitis	3	20	46.7
- meningeoencephalitis	4	26.7	73.3
&pneumonia	2	13.3	86.7
-all except theileriosis			
	1	6.7	93.3

	1	6.7	100
total	15	100	
Most popular drugs :			
-tylosin			
-oxytetracyclin 20%	1	6.7	6.7
-tylosin,penicillin &	5	33.3	40
oxytetracyclin 20%	1	6.7	46.7
- penicillin &			
oxytetracyclin 20%	2	13.3	60
- tylosin,penicillin ,			
oxytetracyclin 20%&	2	13.3	73
sulpha			
-butalex,oxy20%&vits			
-butalex,oxy20%			
,phenylject	1	6.7	80
-oxy &others	1	6.7	86.7
	2	13.3	100
total	15	100	
Last period using drugs:			
-before weeks	2	13.3	13.3
-before a few days	2	13.3	26.7
-not remember	11	73.7	100
total	15	100	
Milking during therapeutics :			
-no	11	73.3	73.3
-yes	4	26.7	100
total	15	100	
Withdrawal period:			
-unknown about it	2	13.3	13.3

-know about it	13	86.7	100.0
total	15	100	
If know, actions they do:			
-discard milk	13	86.7	86.7
-selling for consumption	2	13.3	100.0
total	15	100	

Table 3.3: frequency table for individual milk samples in kh. State :

Risk factors	Frequency	percent	Cumulative percent
Age:			
- Young (<3 years)	39	42.4	42.4
- Medium (2-3 years)	4	4.3	46.7
- Old (>3years)	49	53.3	100
Total	92	100	
B.Cs:			
- poor	16	17.4	17.4
- moderate	66	71.7	89.1
- good	10	10.9	100
Total	92	100	
Result :			
- -ve	62	67.4	67.4
- +ve	30	32.6	100
Total	92	100	

Table 3.4: frequency table for Bulk milk samples in khartoum State :

Risk factors	Frequency	percent	Cumulative percent
Using any disinfectants or penicillin to preserve the milk?			
- No	80	100	100
- Yes	0		
Total	80	100	
Result :			
- -ve	70	87.5	87.5
- +ve	10	12.5	100
Total	80	100	

Table 3.5: frequency table for sales points milk samples in Khartoum State :

Risk factors	Frequency	percent	Cumulative percent
Type of S.P :			
- Street venders	9	14.1	14.1
- Mini markets	55	85.9	100
Total	64	100	
Duration period witch storing the milk:			
- 8-12 hours	52	81.2	81.2
- 1 day	8	12.5	93.8
- > day	4	6.2	100
Total	64	100	
Result :			

- -ve - +ve	54 10	84.4 15.6	84.4 100
Total	64	100	

Cross tabulation shows the positive result for each group in different localities. From 92 individual milk samples the positive result was 30(32.6%); 2(5.4%), 26(63.4%) and 2(14.3%) in khartoum , Bahry and Omdurman respictively. From 80 bulk milk samples the positive result was 10 (12.5%) ; 7(21.2%) , 2 (7.7%) and 1 (4.8%) in khartoum , Bahry and Omdurman respictively. From 64 sales points the positive result was 10 (15.6%) ; 1(4%), 9(56.25%) and 0(0%) in khartoum , Bahry and Omdurman respictively. (Tables 3.6 , 3.7 , 3.8 , 3.9 & 3.10)

Table 3.6: Cross tabulation for individual milk samples between the result of the test and different localities in kh. State :

result * locality Crosstabulation

			locality			
			kh.	bahr.	omdur.	Total
result	-ve	Count	35	15	12	62
		% within locality	94.6%	36.6%	85.7%	67.4%
	+ve	Count	2	26	2	30
		% within locality	5.4%	63.4%	14.3%	32.6%
Total		Count	37	41	14	92
		% within locality	100.0%	100.0%	100.0%	100.0%

Table 3.7: cross tabulation for individual milk samples between using drugs and the result of the test for each locality .

1- Khartoum :

result * using.drugs Crosstabulation

			using.drugs		
			un use	use	Total
result	-ve	Count	34	1	35
		% within using.drugs	94.4%	100.0%	94.6%
	+ve	Count	2	0	2
		% within using.drugs	5.6%	.0%	5.4%
Total		Count	36	1	37
		% within using.drugs	100.0%	100.0%	100.0%

2- Bahry :

result * using.drugs Crosstabulation

			using.drugs		
			un use	use	Total
result	-ve	Count	14	1	15
		% within using.drugs	36.8%	33.3%	36.6%
	+ve	Count	24	2	26
		% within using.drugs	63.2%	66.7%	63.4%
Total		Count	38	3	41
		% within using.drugs	100.0%	100.0%	100.0%

3- Omdurman :

result * using.drugs Crosstabulation

			using.drugs		
			un use	use	Total
result	-ve	Count	10	2	12
		% within using.drugs	83.3%	100.0%	85.7%
	+ve	Count	2	0	2
		% within using.drugs	16.7%	.0%	14.3%
Total		Count	12	2	14
		% within using.drugs	100.0%	100.0%	100.0%

Table 3.8: cross tabulation for bulk milk samples between the result of the test and different localities in Khartoum state :

locality * resulting Crosstabulation

			resulting		
			-ve	+ve	Total
locality	kh	Count	26	7	33
		% within locality	78.8%	21.2%	100.0%
		% within resulting	37.1%	70.0%	41.2%
	bahry	Count	24	2	26
		% within locality	92.3%	7.7%	100.0%
		% within resulting	34.3%	20.0%	32.5%
	omdur	Count	20	1	21
		% within locality	95.2%	4.8%	100.0%
		% within resulting	28.6%	10.0%	26.2%
Total		Count	70	10	80
		% within locality	87.5%	12.5%	100.0%
		% within resulting	100.0%	100.0%	100.0%

Table 3.9: cross tabulation for sales points milk samples between the result of the test and different localities in khartoum state :

the.result * locality Crosstabulation

			locality			
			kh	bahry	omdur	Total
the.result	-ve	Count	24	7	23	54
		% within the.result	44.4%	13.0%	42.6%	100.0%
	+ve	Count	1	9	0	10
		% within the.result	10.0%	90.0%	.0%	100.0%
Total		Count	25	16	23	64
		% within the.result	39.1%	25.0%	35.9%	100.0%

Table 3.10: cross tabulation between using drugs in individual milk sample and location :

Crosstab

			locality			
			kh.	bahr.	omdur.	Total
using.drugs	un use	Count	36	38	12	86
		% of Total	39.1%	41.3%	13.0%	93.5%
	use	Count	1	3	2	6
		% of Total	1.1%	3.3%	2.2%	6.5%
Total		Count	37	41	14	92
		% of Total	40.2%	44.6%	15.2%	100.0%

Univariate analysis by chi-square at ($p<0.25$) shows that , there is a highly significant difference between individual milk samples in different location in khartoum State (0.000) , sales points in different location in khartoum State (0.000) and in bulk milk samples (0.136). (Table 3.11).

Table 3.11: Univariate analysis to determine the prevalence from individual milk samples, bulk milk and sales points in different localities in khartoum State :

Risk factors	total	No. +ve (%)	d.f	Value	p-value
1- individual milk sample - location: 1.kh. 2.bahry 3.omdur.	92 37 41 14	 2(5.4%) 26(63.4%) 2(14.2%)	2	32.3	0.000
2- bulk milk sample - location:	80				

1.kh. 2.bahry 3.omdur.	33 26 21	7(21.2%) 2(7.6%) 1(4.7%)	2	3.98	0.136
3- sales points milk sample - location: 1.kh. 2.bahry 3.omdur.	64 25 16 23	 1(4%) 9(56.2%) 0(0%)	 2	 26.85	 0.000
4- using drugs for individual samples - location: 1.kh. 2.bahry 3.omdur.	6 1 3 2	 0 (0%) 2 (66.6%) 0 (0%)	 2	 2.31	 0.315

In Multivariate analysis p-value set at (<0.05) shows that khartoum is the least affected (Exp B) and highly sig. diff. between other localities (0.000). Then, Bahry was the highest affected (Exp B =10.24) times than Omdurman and p(0.005) , then Omdurman affected (Exp B =0.33)times than khartoum. (Table 3.12).

Table 3.12: Multivariate analysis:

Risk factors	No+ve (%)	Exp (B)	95% CI		p-value
			lower	Upper	
1- Individual milk samples :					
- using drugs.	2 (33.3)	1.30	0.165	10.35	0.7
- Khartoum.	2 (5.4)	_	_	_	0.000
- Omdurman.	2 (14.3)	0.333	0.042	2.659	0.300
- Bahry.	26 (63.4)	10.245	2.008	52.260	0.005
2- Bulk milk samples :					
- Omdurman.					
- Bahry.	1 (4.8)	_	_	_	0.171
- Khartoum.	2 (7.7)	5.385	0.612	47.390	0.129
	7 (21.2)	1.667	0.141	19.758	0.686
3- Sales Points milk samples :					
- Omdurman.	0 (0)	_	_		1.000
- Khartoum.	1 (4)	1.077	0.000	_	0.998
- Bahry.	9 (56.25)	1.000	0.000	_	1.000

In Final Logistic Regression shows the risk factor individual milk sample there highly sig differences between khartoum (p 0.000) and Bahry (p 0.005) and Exp B for Bahry shows it's highest affect 10.24 times than khartoum. (Table 3.13).

Table 3.13: Final Logistic Regression :

Risk factors	No+ve (%)	Exp (B)	95% CI		p-value
			lower	upper	
Individual milk samples :					
- Khartoum.	2 (5.4)	_	_	_	0.000
- Bahry.	26 (63.4)	10.245	2.008	52.260	0.005

In One Way ANOVA p-value set at (≤ 0.05) shows the risk factors individual and sales points are highly sig. diff. (0.000)between there localities.

The high mean was found in the individual milk samples in (Bahry Mean : 0.63 ± STD: 0.48), (Omdurman Mean : 0.14 ± STD:0.36) and (khartoum Mean : 0.05 ± STD: 0.22) with high significant difference (p-value 0.000) . Then, in the bulk milk samples khartoum was the higest mean (khartoum Mean : 0.21 ± STD: 0.41) then (Bahry Mean : 0.07 ± STD: 0.27)and (Omdurman Mean : 0.04 ± STD:0.21) with (p-value 0.140). Sales Points was higher in (khartoum Mean : 0.40 ± STD: 0.50) while Bahry and Omdurman was (Bahry Mean : 0.00 ± STD: 0.00), (Omdurman Mean : 0.00 ± STD:0.00) respectively with high significant difference (p-value 0.000) . (Table 3.14).

Table 3.14: one way ANOVA to determine the significant difference betweens farms in Khartoum State for each group and mean±SD for each localities :

Risk factor	Khartoum	Bahry	Omdurman	p-value
1- Farms :				
- most popular disease	2.16 ± 2.48	3.66±2.16	5.66±4.16	0.227
- most popular drugs	2.16± 2.22	6.33±3.38	5.66±4.50	0.102
- milking during therapeutics	0.33±0.51	0.33±0.51	0.00±0.00	0.564
- action they do	0.83±0.75	1.00±0.89	2.00±0.00	0.120
2- Individual milk samples	0.05±0.22	0.63±0.48	0.14±0.36	0.000
3- Bulk milk samples	0.21±0.41	0.07±0.27	0.04±0.21	0.140
4- Sales points	0.40±0.50	0.00±0.00	0.00±0.00	0.000

Figures

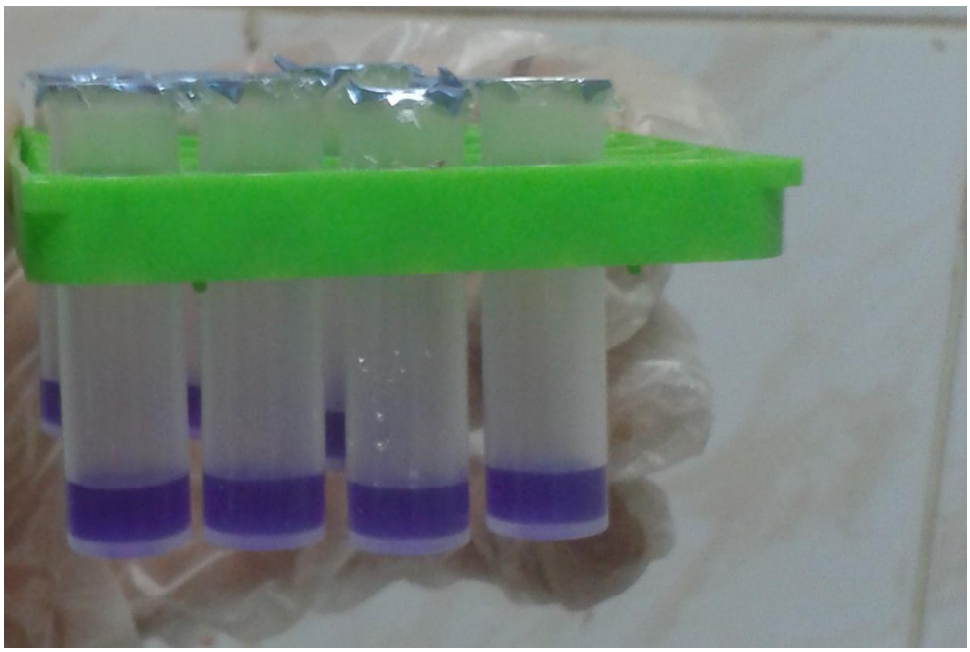

Figure 3.1: The original media color for Delvotest violet color.

Figure 3.2: Delvotest result after 3 hr. incubation period (yellow color indicates –ve result and purple color indicates +ve color).

Figure 3.3: positive and negative result from bottom side.

Figure 3.4: positive result .

List of Abbreviations

ADI: Acceptable Daily Intake.

B.Cs: Body Condition Score.

CAC: Codex Almentarius Commission.

CI 95%: Confidence Interval 95%.

d.f: degree of freedom.

DNA: Dioxy Ribo nucleic acid.

ELISA: Enzyme Linked Immunosorbent Assay.

FAO: Food Additives Organization.

FDA: Food and Drug Agency.

F.P.T: Four Plates Test.

HPLC: High Performance Liquid Chromatography.

JECFA: The Joint Expert Committee on Food Additives.

m RNA: messenger ribo nucleic acid.

MRL: Maximum Residue Limits.

MRLVD: Maximum Residue Limits of Veterinary Drug.

OPT: One Plate Test.

SPSS: Statistical Package for Social Science Program.

STD: Slandered deviation.

SUST: Sudan University for Science and Technology.

t RNA: transport ribo nucleic acid.

WHO: World Health Organization.

CHAPTER FOUR

Discussion

In the present study, 236 milk samples from khartoum state were screened for the presence of antibiotic residues using Delvotest® SP-ampule. The results showed that 21.18% of the milk samples were positive for antibiotic residues. Delvotest® SP-ampule is used widely as screening test for antibiotic residues(Ammar, 2006).

This percentage was neerly agreement with Dinki, 2013 in Guwahati, India who found that 23.3% was positive to antibiotic residues and agree with Roosita who found that 27.78% of samples was positive to antibiotic residues.

Moreover, the present result indicated that the percentage of antibiotic residues in milk samples was high (21.18%) compared to similar studies in other countries , such as Nairobi, Kenya and Washington . Chewulukei (1987) found that 15% of samples in Nairobi, Kenya, contained antimicrobial inhibitors. Another study in Nairobi indicated that all samples were free of any inhibitors (Ombui, 1994), while in Washington, D.C. the percentage of positive samples were 0.03% in 1994 (Smuker, 1996). These low detected levels in these countries may relate to the high level of awareness among milk producers . By contrast the bad agricultural practices and wrong uses of veterinary drugs may be behind the high levels of antibiotic residues .

Moreover, the low level of awareness towards the veterinary drugs withdrawal period after animal treatment may lead to increase the levels of antibiotic residues in milk. This idea was also confirmed by he previous studies showing the situation of veterinary drugs residues in other animal products in sudan (Tasneem, 2006).

These results were different from that reported by Abdulrahman (2001) who reported that there was no antibiotic residues in any of the 110 samples who collected randomly from groceries . And it's higher than Barakat (1995) as he found 8.7% of the milk samples from khartoum state were contaminated by antibiotic. Similarly El- Sherbini and El-sayed (1993) used the Delvotest® P test for the presence of antibiotic residues in 51 raw milk samples in Egypt and found that 7.8% of the examined samples were positive. Tajelsir (2001) found about 10.7% of milk samples positive to antibiotic residues . Ibtisam El Zubair, (2009) reported that 12.25% of milk samples were found to be contaminated with antibiotic.

The percentage of this study was lowest than (Ammar,2006) who reported that 38.9% of examined milk samples were positive by using Delvotest® SP-ampule kit , and (Tasneem,2006) found that 30.9% were positive to antibiotic residues. Moreover, Adil *et.al.,*(2012) found that 33.1% of milk samples were positives by using also Delvotest® kit. Other studies in Indonesia found that 27.78% of samples were positive, Roostita *et.al.,*. Abdul Samad ,2014 in bakistan ,Sindh province observed that among the total of 400 samples of milk, about 49.75% were found to be positive for antibiotic residues. Dinki, (2013) in Guwahati, India found that 23.3% were positive to antibiotic residues. Maha ,2012 found that 100% of the samples are positive with Neomycin and Tylosin in high concentration levels . Hoyda, 2006 also found that the concentrations of the drug residues in milk samples were higher 100% than MRL and Zwald *et.al.,*2004 showing that 80% of the conventional herds are contaminated with antibiotic residues.

The results of antibiotic detection in raw milk samples from individual showed 32.6% , sales points 15.6% and from bulk milk 12.5% when comparing with Ammar, 2006 lowest in 22.2 %from farms milk samples are positive and higest in 55.6% from sales points. And it's lowest than Adil *et.al.,*2012 who repoted 42.4% from farms are positive and lowest in sales points that 23.2% are positive. But Mahmood, 2001 higher than this result in bulk , sales points and lowest in farms (21.7%, 21.6% and 9.1%) respectively.

Antibiotic residues percentage were detected in Khartoum 20%, Bahry 74% and in Omdurman 6% comparing with other studies in Khartoum State Ammar ,2006 who founds 23.6% in Khartoum and 15.3% in Bahry. Tasneem, 2006 founds that 26.6 % in Khartoum, 30.5% in Bahry and 35 % in Omdurman. Hoyda,2006 found that the highest incidence was recorded in Bahry 54.8% compared with Khartoum 23.2% and Omdurman 22%.

The high mean was found in the individual milk samples in (Bahry Mean : 0.63 ± STD: 0.48), (Omdurman Mean : 0.14 ± STD:0.36) and (khartoum Mean : 0.05 ± STD: 0.22). then, in the bulk milk samples khartoum was the higest mean (khartoum Mean : 0.21 ± STD: 0.41) then (Bahry Mean : 0.07 ± STD: 0.27)and (Omdurman Mean : 0.04 ± STD:0.21). sales points was higher in (khartoum Mean : 0.40 ± STD: 0.50) while Bahry and Omdurman was (Bahry Mean : 0.00 ± STD: 0.00), (Omdurman Mean : 0.00 ± STD:0.00) respectively. That compared with Maha , 2012 who studied both neomycin and tylosin and found the highest mean in (khartoum Mean : 3.4315 ± STD: 0.288890), (Bahry Mean : 3.0952 ± STD: 32273)and (Omdurman Mean : 34732± STD:0.34732) respectively.

The season of collection may also affect these results to some extent due to the high rate of infections with inflammation during the rainy season. In addition to that , methods followed in production and distribution in sudan are so poor, the dealers tend to use means to prolong the shelflife of milk even if it is not permissible especially in the absence of monitoring programmes. (Tasneem,2006) .

The tetracyclines group was found to be the most commonly used antibiotic in khartoum state . They were reported by (Koenen – Dierick *et.al.,* 1998), To be easily detected using microbiological assays by the one plate method.

More monitoring for antibiotic residues is needed to explain the observation of high percentage of positive samples. More attention to the withdrawal time by veterinary practitioners in khartoum state is also required because mass treatment in livestock will continue to treat disease conditions which are related to low hygiene of environmental surroundings. (Nazik, 1999).

The residues of antibiotics are harmful to humans, resulting into therapy failure and development of antibiotic resistant organisms (shitandi *et.al,* 2004) (El zubeir, *et.al,* 2006) (yagoub *et.al.,* 2005). Moreover, antibiotic residues in milk are undesirable for public health and for technological reasons (booth, 1998).

The presence of antibiotics in the milk samples of most of the studied farms might be due to the fact that during winter when weather becomes cold diseases such as pneumonia increased and farmers used antibiotics to treat animals , therefore antibiotics residues transferred into milk. Also, it might indicate the increase of awareness among the animal owners, which could be attributed to the increased education levels and increased veterinarian visits during animal treatment . The high awareness was also observed from our informal discussion with some of the farmers and milk sellers who supply clean milk.

Conclusion and Recommendations

CONCLUSION:

The introduction of antimicrobial residue into milk chain can be mainly from dairy animals in which it is applied for treatment purpose, as food additives and as growth promoter.

1. Generally antimicrobial/antibiotics residue which is beyond the Maximum Residue Limit (MRL) can cause adverse effect on public health, diary industry and environment.
2. The proper choice of antibiotic screening test plays an important role in the effectiveness and accuracy of residue detection.
3. Screening tests are used to prevent the introduction of the contaminated milk into food chain and therefore they are frequently used by regulators and food producers.
4. Screening tests can decrease the danger of residue contaminations at violative levels if they are reliable to detect them at the concentrations found in bulk and tanker truck milk.
5. The regulatory bodies should be formed and control the antimicrobial residue limit before consumption (screening and quantitative evaluation of the level of antibiotic residue in milk).
6. Sampling and testing protocols should be designed and properly applied.

Recommendations:

1. Sustainable veterinary supervision milk production in farms should be established through well- trained veterinarians.
2. Effective surveillance, monitoring and control on the use of veterinary drugs should be performed to prevent drug residues in animal products.
3. Increase the awareness of the milk producers' distributors and consumer towards the hazard of the improper uses of antibiotic and their right to consume safe and healthy foods.
4. Government should establish efficient inspection and detection programmes .

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Appendices

Appendix A

Statistics

		herd	health.status	most.pop.dis	most.pop.drug	last.per.u.d	milk.dur.therap	withdrawal period	if know about it , actions they do :
N	Valid	15	15	15	15	15	15	15	15
	Missing	0	0	0	0	0	0	0	0
Mean		.8000	.9333	3.4667	4.5333	1.6000	.2667	.8667	.1333
Std. Deviation		.67612	.25820	2.85023	3.58303	.73679	.45774	.35187	.35187
Percentiles	25	.0000	1.0000	1.0000	1.0000	1.0000	.0000	1.0000	.0000
	50	1.0000	1.0000	3.0000	5.0000	2.0000	.0000	1.0000	.0000
	75	1.0000	1.0000	7.0000	8.0000	2.0000	1.0000	1.0000	.0000
	100	2.0000	1.0000	9.0000	10.0000	2.0000	1.0000	1.0000	1.0000

Herd

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	small	5	33.3	33.3	33.3
	medium	8	53.3	53.3	86.7
	large	2	13.3	13.3	100.0
	Total	15	100.0	100.0	

health.status

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	poor	1	6.7	6.7	6.7
	good	14	93.3	93.3	100.0
	Total	15	100.0	100.0	

most.pop.dis

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	meningioencephalitis	1	6.7	6.7	6.7
	pneumonia	3	20.0	20.0	26.7
	mastitis	3	20.0	20.0	46.7
	theileriosis	4	26.7	26.7	73.3
	pneumonia & mastitis	2	13.3	13.3	86.7
	meningioencephalitis & pneumonia	1	6.7	6.7	93.3
	all except theileriosis	1	6.7	6.7	100.0
	Total	15	100.0	100.0	

most.pop.drug

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	tylosin	1	6.7	6.7	6.7
	oxytetracyclin 20%	5	33.3	33.3	40.0
	tylosin, penicillin & oxytetracyclin	1	6.7	6.7	46.7
	penicillin & oxy tetraccyclin 20%	2	13.3	13.3	60.0
	tylosin , penicillin, oxy. & sulpha	2	13.3	13.3	73.3
	butalex , oxy20% & vitamins	1	6.7	6.7	80.0
	butalex, oxy. , phenylject	1	6.7	6.7	86.7
	oxy & others	2	13.3	13.3	100.0
	Total	15	100.0	100.0	

last.per.u.d

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	before weeks	2	13.3	13.3	13.3
	before a few days	2	13.3	13.3	26.7
	not remember	11	73.3	73.3	100.0
	Total	15	100.0	100.0	

milk.dur.therap

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	no	11	73.3	73.3	73.3
	yes	4	26.7	26.7	100.0
	Total	15	100.0	100.0	

withdrawal period

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	unknown about it	2	13.3	13.3	13.3
	known about it	13	86.7	86.7	100.0
	Total	15	100.0	100.0	

if know about it , actions they do :

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	discard milk	13	86.7	86.7	86.7
	selling for consumption	2	13.3	13.3	100.0
	Total	15	100.0	100.0	

Appendix B

Statistics

		age	Body condition scor	result
N	Valid	92	92	92
	Missing	0	0	0
Percentiles	100	2.0000	2.0000	1.0000

age

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	young (< 3 years)	39	42.4	42.4	42.4
	medium (2-3 years)	4	4.3	4.3	46.7
	old (> 3 years)	49	53.3	53.3	100.0
	Total	92	100.0	100.0	

Body condition scor

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	poor	16	17.4	17.4	17.4
	moderate	66	71.7	71.7	89.1
	good	10	10.9	10.9	100.0
	Total	92	100.0	100.0	

result

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	62	67.4	67.4	67.4
	+ve	30	32.6	32.6	100.0
	Total	92	100.0	100.0	

result * locality Crosstabulation

			locality			
			kh.	bahr.	omdur.	Total
result	-ve	Count	35	15	12	62
		% within locality	94.6%	36.6%	85.7%	67.4%
	+ve	Count	2	26	2	30
		% within locality	5.4%	63.4%	14.3%	32.6%
Total		Count	37	41	14	92
		% within locality	100.0%	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	32.304[a]	2	.000
Likelihood Ratio	35.278	2	.000
Linear-by-Linear Association	5.595	1	.018
N of Valid Cases	92		

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.57.

Descriptives

result								
					95% Confidence Interval for Mean			
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
kh.	37	.0541	.22924	.03769	-.0224-	.1305	.00	1.00
bahr.	41	.6341	.48765	.07616	.4802	.7881	.00	1.00
omdur.	14	.1429	.36314	.09705	-.0668-	.3525	.00	1.00
Total	92	.3261	.47135	.04914	.2285	.4237	.00	1.00

ANOVA

result

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.099	2	3.550	24.081	.000
Within Groups	13.118	89	.147		
Total	20.217	91			

Multiple Comparisons

result

LSD

(I) locality	(J) locality	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kh.	bahr.	-.58009*	.08706	.000	-.7531-	-.4071-
	omdur.	-.08880-	.12047	.463	-.3282-	.1506
bahr.	kh.	.58009*	.08706	.000	.4071	.7531
	omdur.	.49129*	.11884	.000	.2552	.7274
omdur.	kh.	.08880	.12047	.463	-.1506-	.3282
	bahr.	-.49129*	.11884	.000	-.7274-	-.2552-

*. The mean difference is significant at the 0.05 level.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	using.drugs(1)	.268	1.056	.065	1	.799	1.308	.165	10.355
	locality			23.088	2	.000			
	locality(1)	-1.099-	1.060	1.076	1	.300	.333	.042	2.659
	locality(2)	2.327	.831	7.832	1	.005	10.245	2.008	52.260
	Constant	-2.025-	1.203	2.835	1	.092	.132		

a. Variable(s) entered on step 1: using.drugs, locality.

Appendix C

		using any disinfectants or penicillin to preserve the milk?	resulting
N	Valid	80	80
	Missing	0	0
Percentiles	100	.0000	1.0000

using any disinfectants or penicillin to preserve the milk?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	no	80	100.0	100.0	100.0

resulting

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	70	87.5	87.5	87.5
	+ve	10	12.5	12.5	100.0
	Total	80	100.0	100.0	

locality * resulting Crosstabulation

			resulting		Total
			-ve	+ve	
locality	kh	Count	26	7	33
		% within locality	78.8%	21.2%	100.0%
		% within resulting	37.1%	70.0%	41.2%

	bahry	Count	24	2	26
		% within locality	92.3%	7.7%	100.0%
		% within resulting	34.3%	20.0%	32.5%
	omdur	Count	20	1	21
		% within locality	95.2%	4.8%	100.0%
		% within resulting	28.6%	10.0%	26.2%
Total		Count	70	10	80
		% within locality	87.5%	12.5%	100.0%
		% within resulting	100.0%	100.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.989[a]	2	.136
Likelihood Ratio	4.035	2	.133
Linear-by-Linear Association	3.502	1	.061
N of Valid Cases	80		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 2.63.

Descriptives

resulting

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
kh	33	.2121	.41515	.07227	.0649	.3593	.00	1.00
bahry	26	.0769	.27175	.05329	-.0328-	.1867	.00	1.00
omdur	21	.0476	.21822	.04762	-.0517-	.1470	.00	1.00
Total	80	.1250	.33281	.03721	.0509	.1991	.00	1.00

ANOVA

resulting

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.436	2	.218	2.021	.140
Within Groups	8.314	77	.108		
Total	8.750	79			

Multiple Comparisons

resulting

LSD

(I) locality	(J) locality	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kh	bahry	.13520	.08617	.121	-.0364-	.3068
	omdur	.16450	.09172	.077	-.0181-	.3471
bahry	kh	-.13520-	.08617	.121	-.3068-	.0364
	omdur	.02930	.09641	.762	-.1627-	.2213
omdur	kh	-.16450-	.09172	.077	-.3471-	.0181
	bahry	-.02930-	.09641	.762	-.2213-	.1627

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	locality			3.530	2	.171			
	locality(1)	1.684	1.110	2.302	1	.129	5.385	.612	47.390
	locality(2)	.511	1.262	.164	1	.686	1.667	.141	19.758
	Constant	-2.996-	1.025	8.547	1	.003	.050		

a. Variable(s) entered on step 1: locality.

Appendix D

		type.S.P	duration.period	the.result
N	Valid	64	64	64
	Missing	0	0	0
Percentiles	100	1.0000	2.0000	1.0000

type.S.P

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	street vender	9	14.1	14.1	14.1
	mini market	55	85.9	85.9	100.0
	Total	64	100.0	100.0	

duration.period

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	8-12 hour	52	81.2	81.2	81.2
	1 day	8	12.5	12.5	93.8
	> day	4	6.2	6.2	100.0
	Total	64	100.0	100.0	

the.result

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	-ve	54	84.4	84.4	84.4
	+ve	10	15.6	15.6	100.0
	Total	64	100.0	100.0	

the.result * locality Crosstabulation

			locality			Total
			kh	bahry	omdur	
the.result	-ve	Count	24	7	23	54
		% within the.result	44.4%	13.0%	42.6%	100.0%
	+ve	Count	1	9	0	10
		% within the.result	10.0%	90.0%	.0%	100.0%
Total		Count	25	16	23	64
		% within the.result	39.1%	25.0%	35.9%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	26.852[a]	2	.000
Likelihood Ratio	25.148	2	.000
Linear-by-Linear Association	.074	1	.786
N of Valid Cases	64		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 2.50.

Descriptives

the.result

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
kh	25	.4000	.50000	.10000	.1936	.6064	.00	1.00
bahry	16	.0000	.00000	.00000	.0000	.0000	.00	.00
omdur	23	.0000	.00000	.00000	.0000	.0000	.00	.00
Total	64	.1562	.36596	.04575	.0648	.2477	.00	1.00

ANOVA

the.result

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.438	2	1.219	12.391	.000
Within Groups	6.000	61	.098		
Total	8.438	63			

Multiple Comparisons

the.result

LSD

(I) locality	(J) locality	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
kh	bahry	.40000*	.10041	.000	.1992	.6008
	omdur	.40000*	.09061	.000	.2188	.5812
bahry	kh	-.40000*	.10041	.000	-.6008-	-.1992-
	omdur	.00000	.10210	1.000	-.2042-	.2042
omdur	kh	-.40000*	.09061	.000	-.5812-	-.2188-
	bahry	.00000	.10210	1.000	-.2042-	.2042

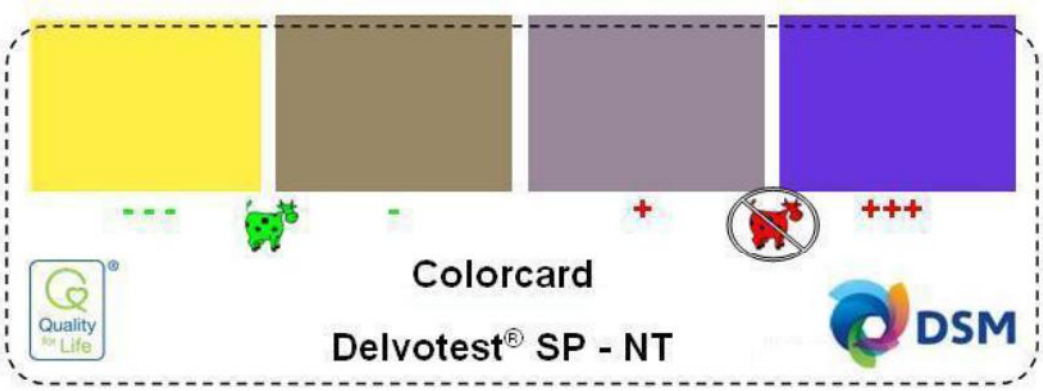
*. The mean difference is significant at the 0.05 level.

Variables in the Equation

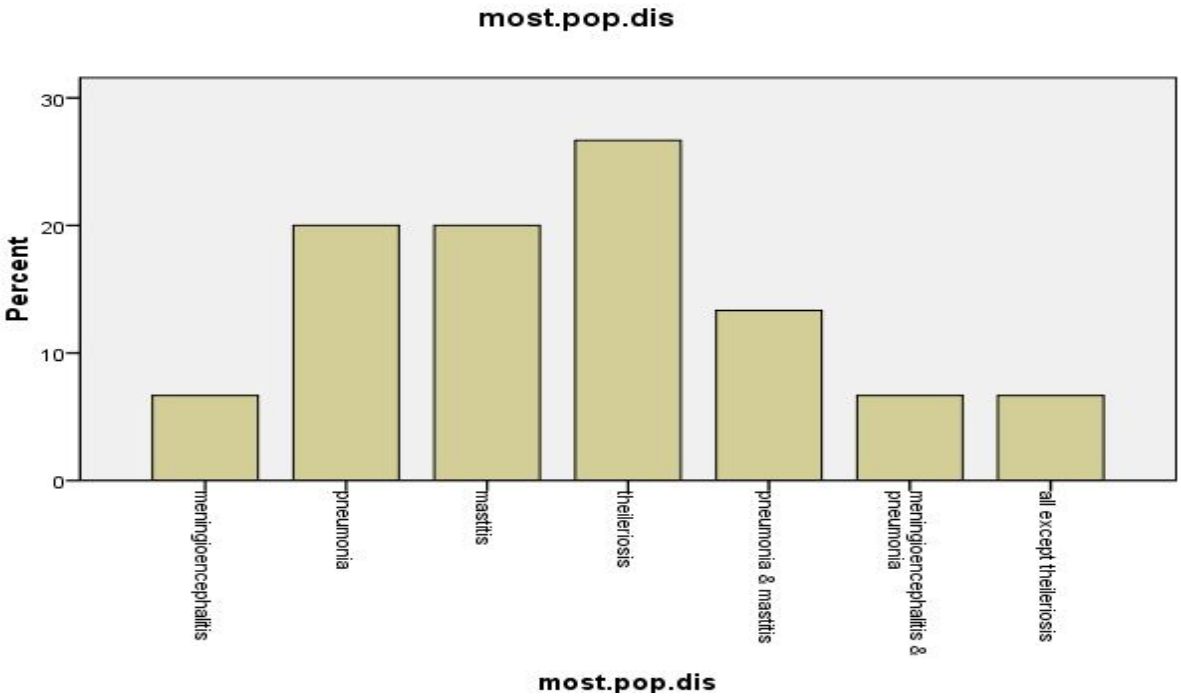
		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	locality			.000	2	1.000			
	locality(1)	20.797	8.381E3	.000	1	.998	1.077E9	.000	.
	locality(2)	.000	1.308E4	.000	1	1.000	1.000	.000	.
	Constant	-21.203-	8.381E3	.000	1	.998	.000		

a. Variable(s) entered on step 1: locality.

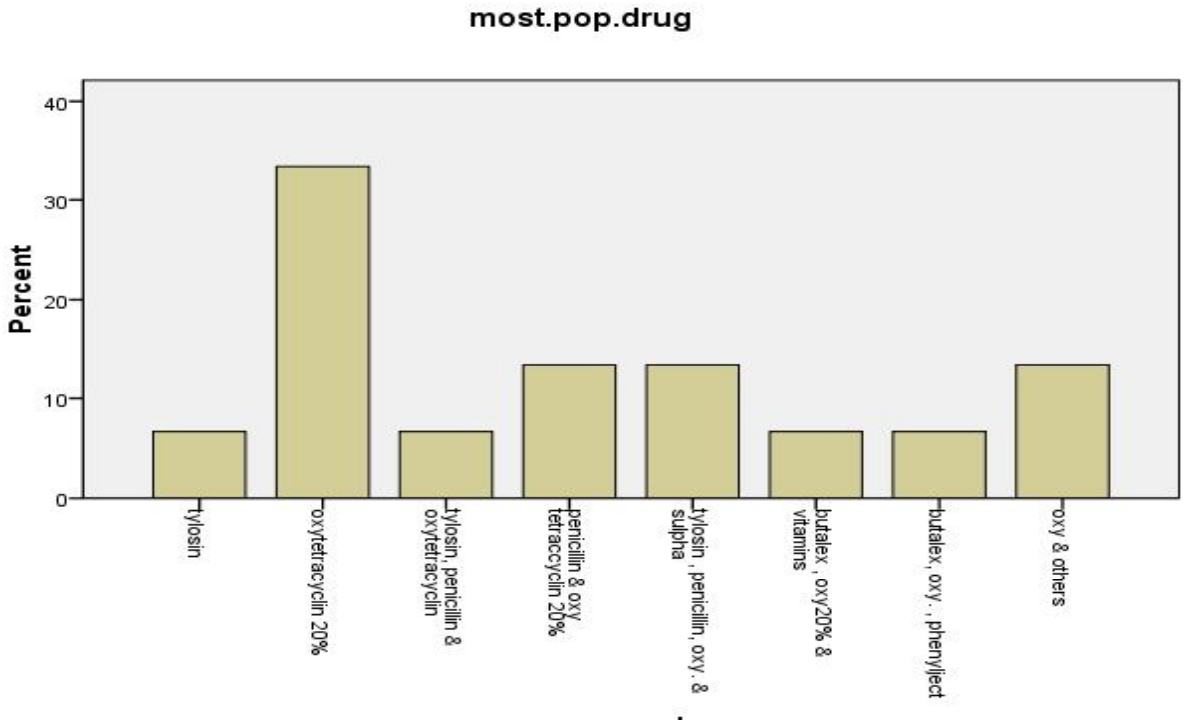
Appendix E



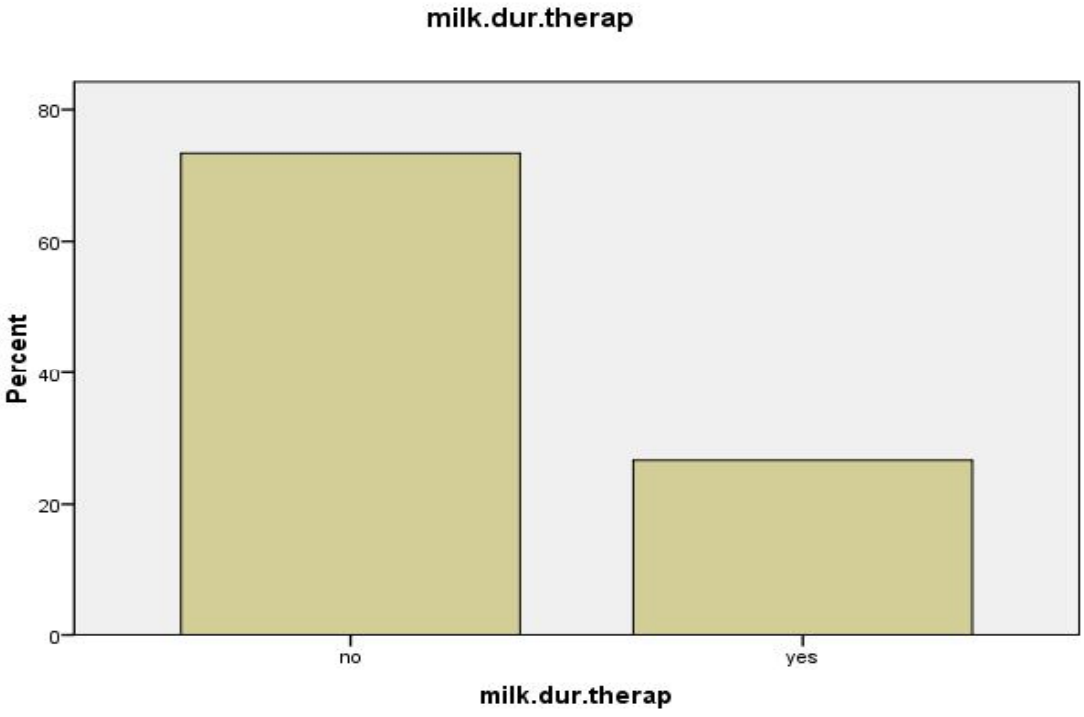
FigureE.1: color card for Delvotest® SP-ampule kits.



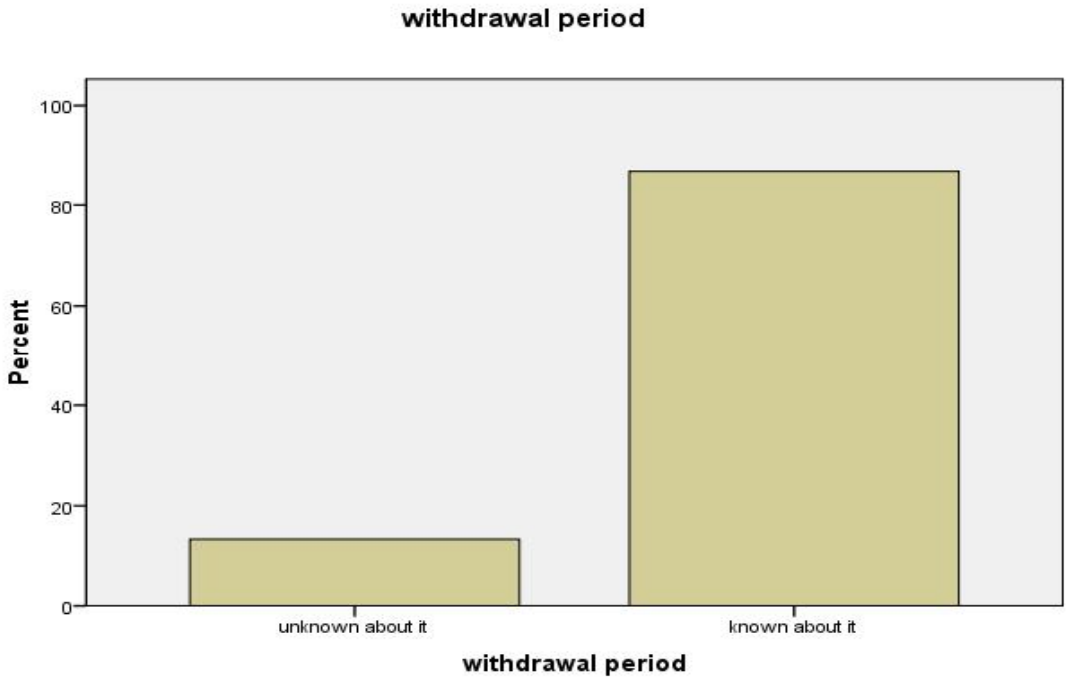
Bar chart E.2 : most popular disease.



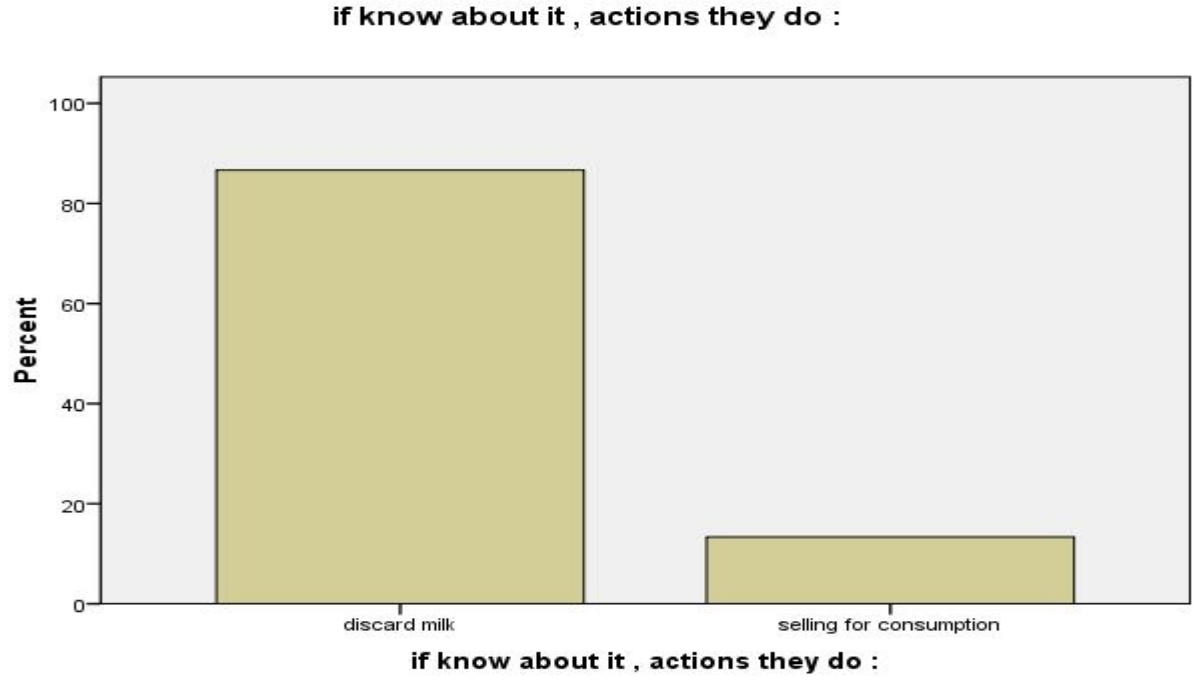
Bar chart E.3: most popular drugs.



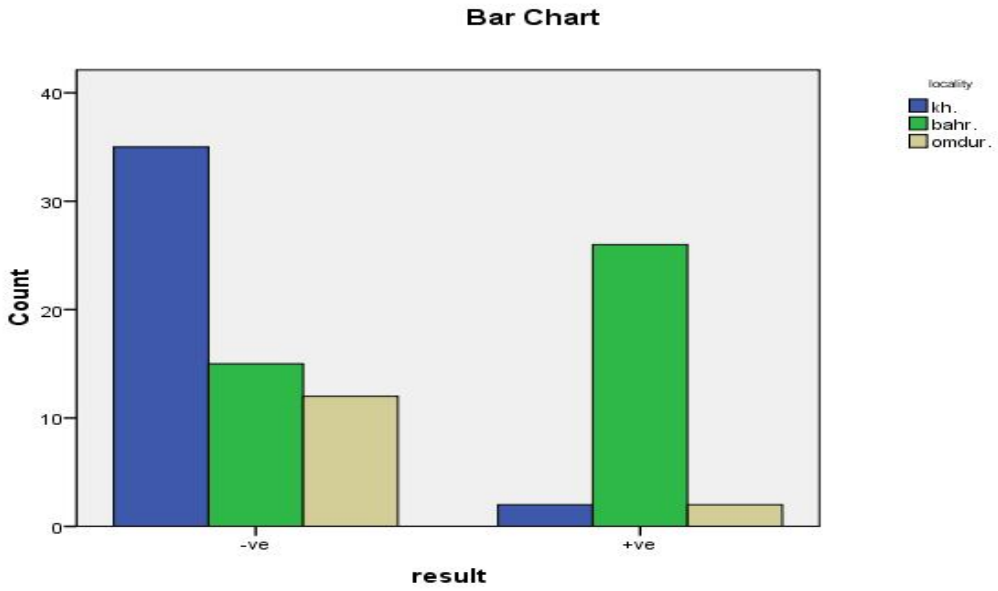
Bar chart E.4: milking during theraputics .



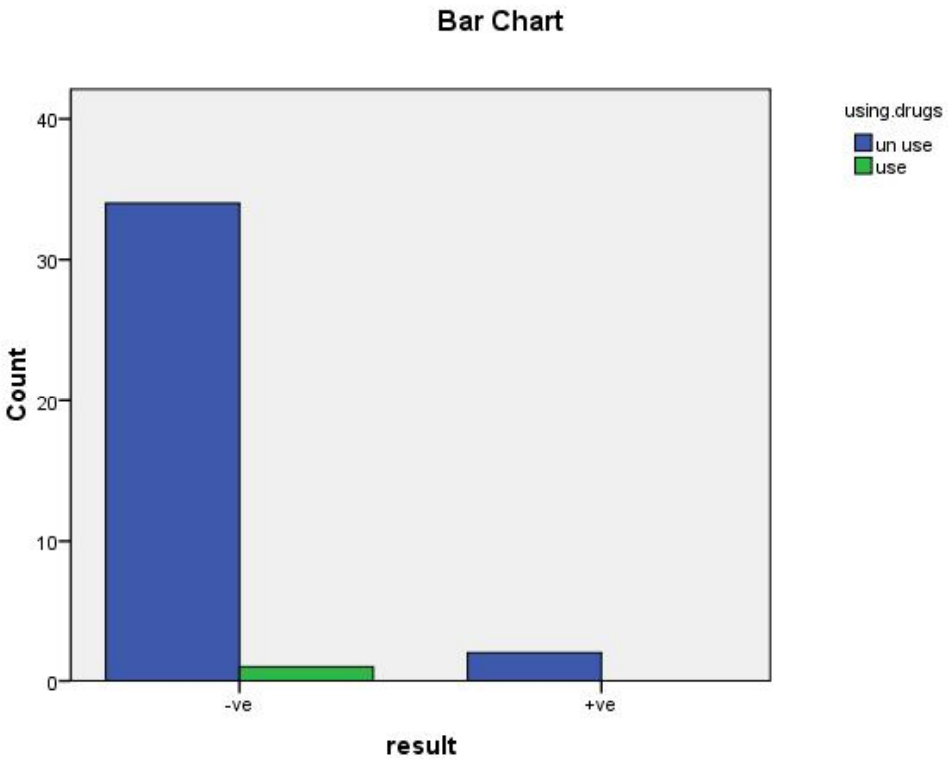
Bar chart E.5: withdrwal period



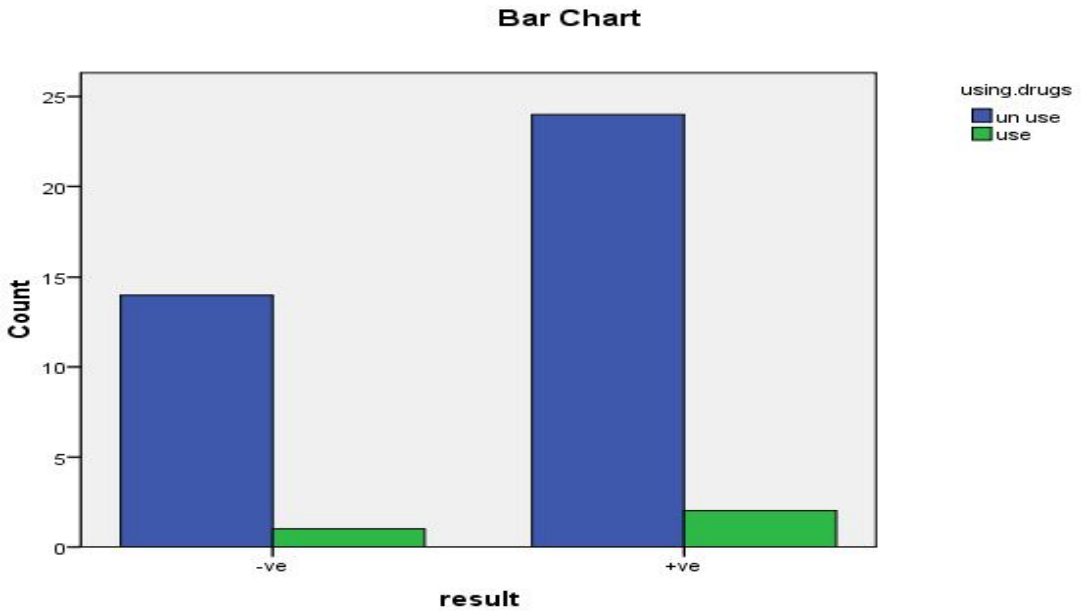
Bar chart E.6: actions they do.



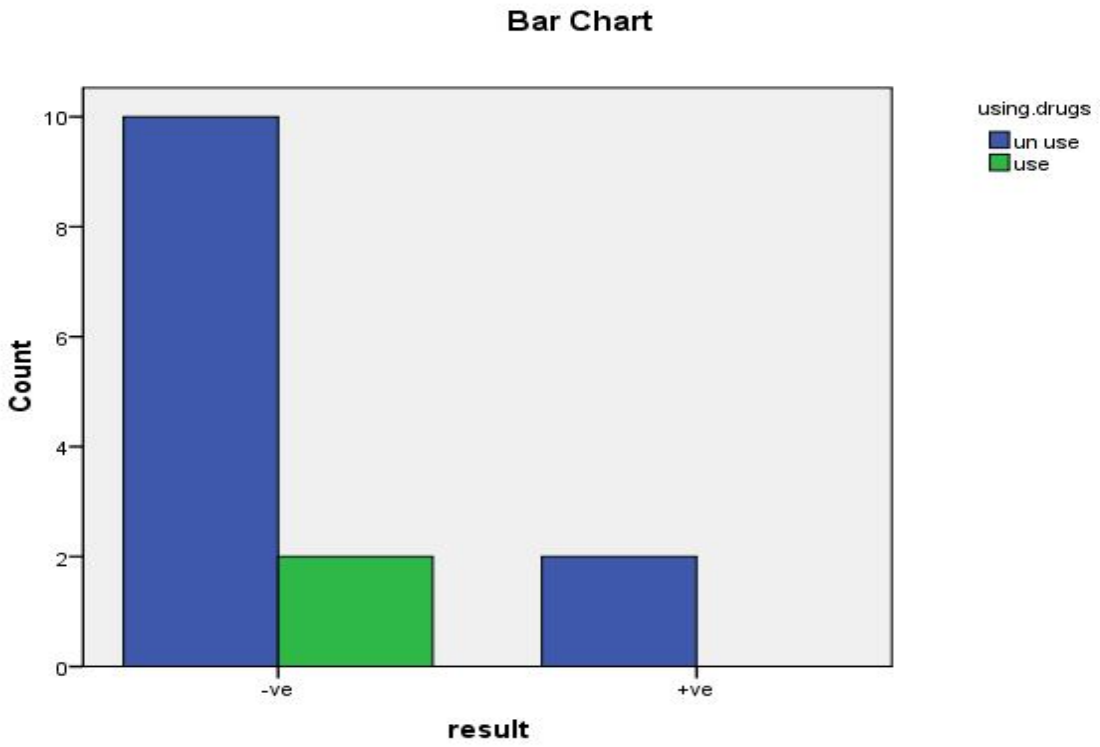
Bar chart E.7: result for individual milk samples in different location in khartoum state .



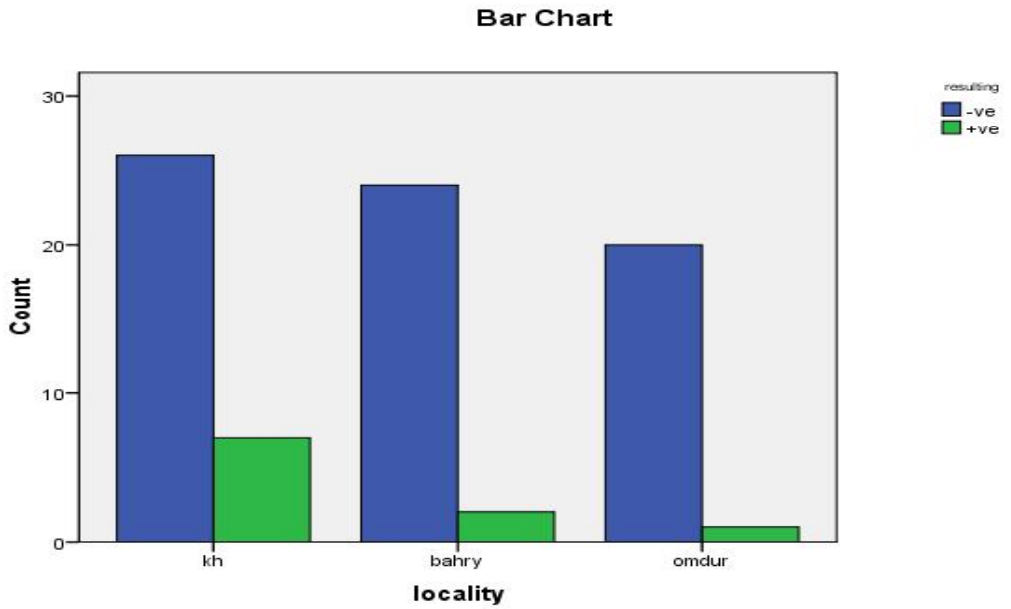
Bar chart E.8: result of using drugs for individual milk sample in khartoum.



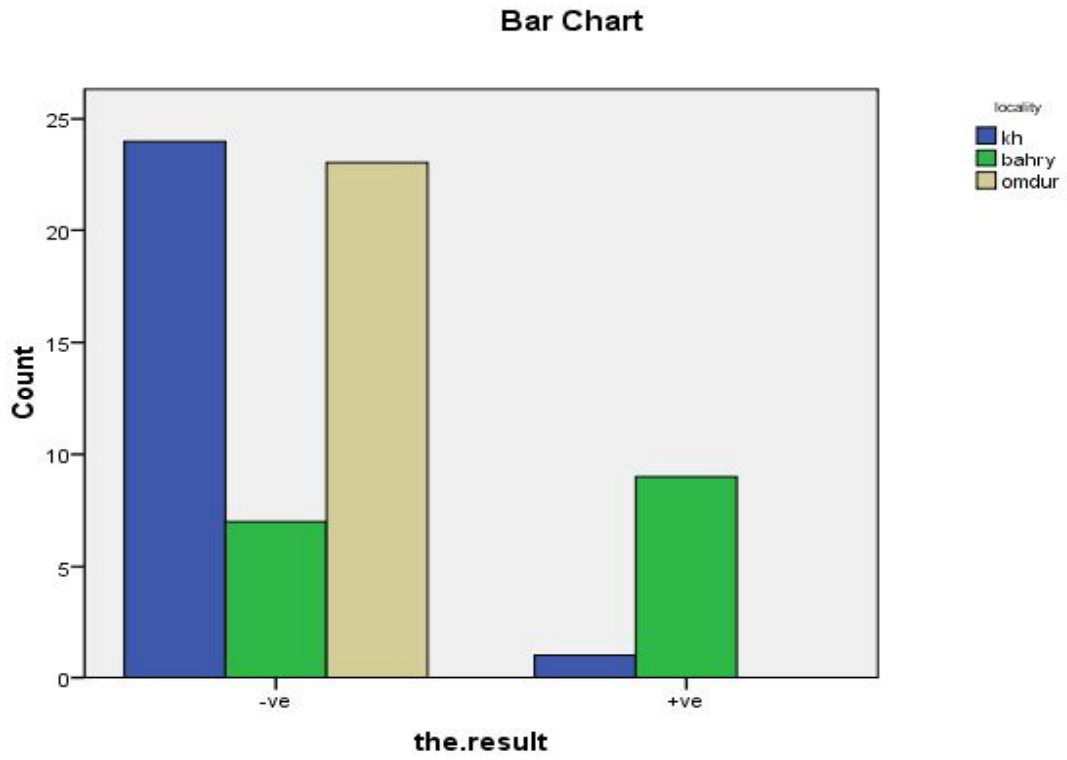
Bar chart E.9: result of using drugs for individual milk sample in Bahry.



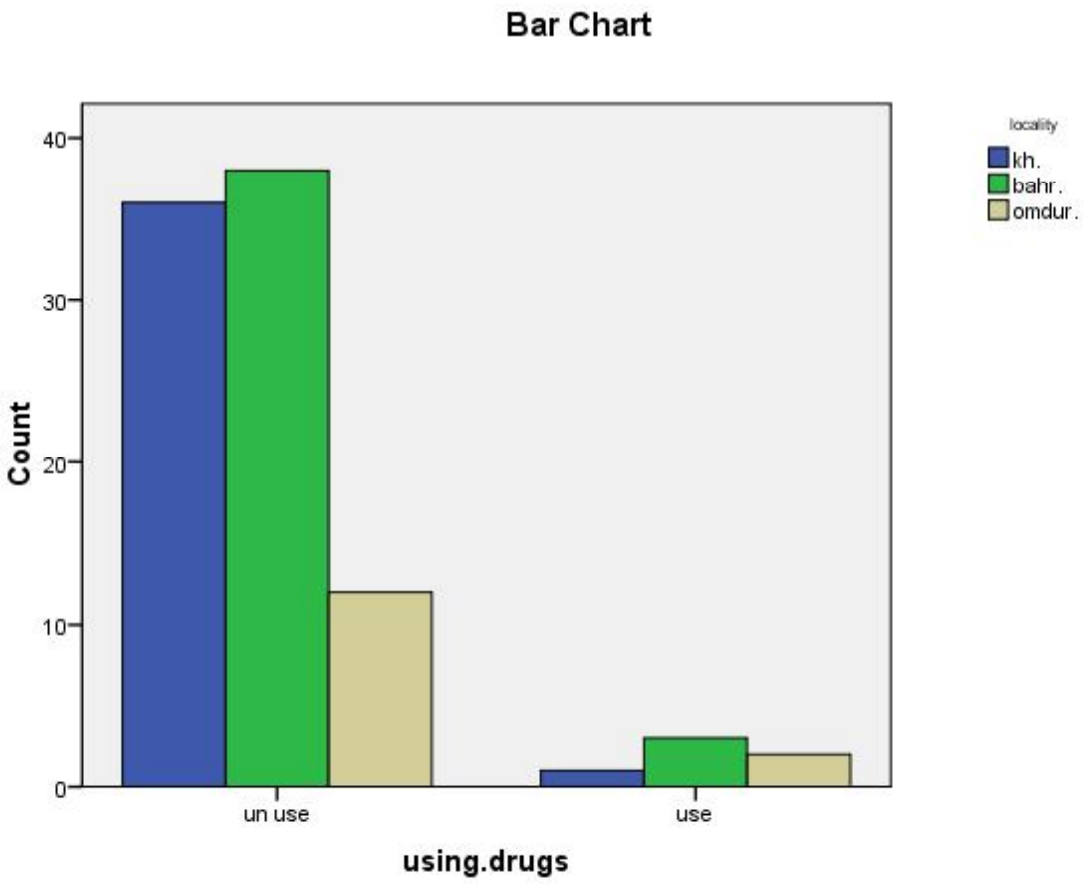
Bar chart E.10: result of using drugs for individual milk sample in Omdurman



Bar chart E.11: result of bulk milk sample in khartoum state.



Bar chart E.12: result of sales points milk samples in khartoum state.



Bar chart E.13: using drugs for individual milk samples in khartoum state.

Appendix F

بسم الله الرحمن الرحيم

Questionnaire

FROM FARMS :

A): Owner's Information:

1. Owner ŝ name, Telephone & Address:
2. Herd size :
3. Health status of heard:
   a. Poor
   b. Good.
4. Most popular disease :
5. Most popular using drugs:
6. Last period using drugs before …
   a. Weeks.
   b. A few days.
7. Milking during therapeutic period?
   a. Yes.
   b. No.
8. Withdrawal period :
   a- know about withdrawal period
   b- unknown about withdrawal period
9. If know about withdrawal period , actions they do :
   a- discard milk
   b- selling for consumption

B): Animals information (individual milk sample):

1. Age :
    a. >1year.
    b. 2-3year.
    c. <3 year.
2. Breed :
    a. Local.
    b. Mix.
3. Body condition score (B.C.s) :
    a. Good.
    b. Moderate.
    c. Poor.
4. Using drugs :
    a. Yes.
    b. No.

    If yes (mention)….

FROM CONTAINERS (BULK MILK):

1. Farmers name and tel. (if there).
2. Source of milk.
3. Using any disinfectants or penicillin to preserve the milk?
    a. Yes.
    b. No.

    If yes (mention them)….

FROM SALES POINTS:

1. Type of sales points :
    a- street vender.
    b- mini markets.
2. Source of milk :
3. Duration period which stores the milk:
    a. 8-12 hours.
    b. 1 day.
    c. > day.

Appendix G

Overview of the indicative detection levels of Delvotest® SP for different antibiotic:

Drugs	**Control time** **Time of negative control** **Colouring yellow**	**Fixed reading time between** **2 hours, 45 minutes and 3hours**
ß-lactams		
Penicillin-G	2	2.5
Ampicillin	*3-2*	*5-3*
Amoxicillin	*2*	*5-3*
Cefacetril	*20*	*40-20*
Cefalexin	*60-40*	*100-60*
Cefalonium	*10-5*	*25-10*
Cefaperazon	*40*	*100-60*
Cefapirin	*5*	*10-5*
Ceftiofur	*<50*	*70-50*
Cloxacillin	*15*	*25-15*

Dicloxacillin	*10*	*15-10*
Oxacillin	*5*	*10*
Nafcillin	*5*	*10-5*
Sulphonamides		
Sulfadiazine	*50*	*100-50*
Sulfadimethoxine	*50*	*100-50*
Sulfamethazine	*25*	*200-50*
Sulfathiazole	*50*	*150-50*
Tetracyclines		
Tetracycline	*100*	*600-200*
Oxytetracycline	*100*	*500-200*
Chlortetracycline	*150-100*	*600-200*
Macrolides		
Erythromycin	*50*	*250-100*
Tylosin	*20-10*	*100-3*
Spiramycin	*200*	*-350ns*
Aminoglycosides		
Gentamycin	*300-100*	*500-200*
Neomycin	*200-100*	*2000-300*

Dihydrostreptomycin	*500-300*	*10000-1500*
Kanamycin	*2500*	*-7500ns*
Lincosamides		
Lincomycin	*100*	*400-200*
Others		
Trimethoprim	*50*	*500-100*
Dapsone	*1*	*8-1*
Chloramphenicol	*2500*	*10000-7500*

* manufacture sheet.

The data indicated in the table are based upon readings judged as being yellow/purple to purple and are to be regarded as an indication.
It is recommended to always confirm positive test results. Variations in incubation
times and temperatures, operators and storage conditions may have an influence on the reading result found.
***Drugs:** Active ingredient of cattle medication such as antibiotics and sulfonamides.

***Spectrum:** An indication of the number of different active ingredients that can be detected by a given method at or below the required detection level.

***Detection level:** The lowest concentration at which a drug is still detected by the test, especially at control time and reading time.

***Control time:** Time at which the test agar containing the negative control sample has just changed to yellow. At this time the best sensitivity for the test system is obtained.

***Fixed Reading time:** For use without the requirement of running a negative control sample a fixed reading time between 2 hours 45 minutes and 3 hours can be used. This may decrease the sensitivity for bacteriostatic drugs.

***ns :** Not sensitive, detection limit above 10000 ppb (ng/ml) (10 □g/ml)